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PHYSIOLOGICAL FEATURES OF ROOTS, WITH ESPECIAL REFERENCE TO THE RELATION OF ROOTS TO AERATION OF THE SOIL

BY

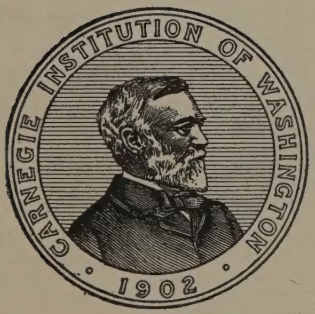
WILLIAM AUSTIN CANNON

WITH A CHAPTER ON

DIFFERENCES BETWEEN NITROGEN AND HELIUM AS INERT GASES IN ANAEROBIC EXPERIMENTS ON PLANTS

BY

EDWARD ELWAY FREE



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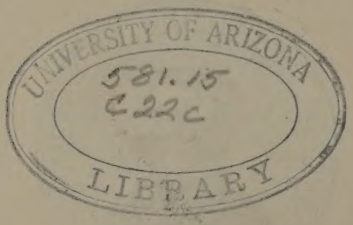

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PHYSIOLOGICAL FEATURES OF ROOTS, WITH ESPECIAL REFERENCE TO THE RELATION OF ROOTS TO AERATION OF THE SOIL[1]

INTRODUCTION.

The relations of aerobic plants to oxygen are of a fundamental nature, diverse, and complex. They have to do not only with physical-physiological-chemical processes within the cell or tissues, with which the present study is not mainly concerned, but with many more obvious reactions, affecting both root and shoot. Moreover, the oxygen relations, intracellular, intercellular, and extracellular, may be dependent on or modified by many environmental factors intimately related to the cell, organ, or plant.

It is self-evident that not all of the organs of multicellular plants, independent of their function, are similarly related to the oxygen-supply. The oxygen relation, like certain other environmental relations, is in part influenced by the medium in which the plant lives, as well as by the morphological nature of the organ, or plant of itself. In the submerged and attached lower plants, both the free distal portion and the attached proximal portion in the adult have essentially similar relations to oxygen. But in such plants as have assumed the land habit, however, the organs of attachment have relations to oxygen which are usually and fundamentally quite different from those of the shoot. Moreover, it is also apparent that where the roots of land plants are extensive, reaching out widely or penetrating deeply, the members of one and the same root system may have unlike relations to oxygen, owing mainly to possible differences in the oxygen-content of different strata of the soil. And, finally, the amount of oxygen in any stratum may vary from time to time from different causes, both climatic and biologic.

A close connection exists between the absorbing cells of the root of a land plant and soil particles, ground water, and soil atmosphere. The water-saturated walls of the root-hairs, for example, are intimately related to the water-films of the soil, with a cell wall-water boundary, but with a continuity of water. A gas-water boundary delimits the water-films on the opposite side. Whatever factors, therefore, operate to modify the oxygen-content of the atmosphere of the soil, induces a related and immediate change in the oxygen-content of the water-films which is further communicated to that of the water-saturated cell-walls, and, finally, to the interior of the cell.

[1] By W. A. Cannon.

In the gas-filled pores of the soil, both molar gaseous movement and diffusion take place, but in the water film-cell wall-cell portion of the gaseous chain, movement is by diffusion only. The direction of the movement of oxygen under conditions that commonly obtain is from the soil atmosphere into and through the water-films of the soil, into and through the outer cell walls of the root, and into the cell interior. Here the oxygen is utilized, and from the outer cells of the root it makes its way to those more deeply placed. A concentration gradient of oxygen may thus exist between the intracellular portion of the gaseous chain and the portion in solution in the water-films outside of the body of the root. This, apparently, may at times, if not always, be very considerable, with the effect that a negative partial pressure may exist in the deeper tissues of the plant and at the same time a relatively low partial pressure of oxygen. Whatever forces work to modify the rate of consumption of oxygen, such as an increase in the rate of growth, disturb the value of the oxygen gradient, which also may be directly and indirectly affected by biological and by physical features of many kinds in the surrounding soil and its atmosphere. It is conceivable, therefore, that the relative oxygen partial pressure in plant and in soil may even be the reverse of that postulated in the foregoing, and that in case of appropriate conditions the movement of oxygen may be in the opposite direction, that is to say, from the plant into and through the water-films of the soil and into the atmosphere of the soil. Or, at any rate, that the gradient of oxygen may approach the neutral point.

The temperature of the soil is a factor of very great importance in influencing the rate of root-growth. At relatively high temperatures the growth-rate is rapid; at relatively low temperatures it is slow. And, at length, where the temperatures are excessively high or low, relatively speaking, growth ceases. Under such circumstances the oxygen-supply is assumed to be normal for the species. In case, however, the partial pressure of oxygen falls greatly below the "normal," the oxygen-supply, and not temperature, determines the growth-rate of roots. This important although indirect effect of temperature, in extremely small concentrations of oxygen, is apparently related to the absorbing capacity of water for the given temperatures.

The interrelation of temperature and the supply of oxygen, as suggested, may influence root-growth in various ways, of which certain ways either have not hitherto been recognized or application has not been made to roots. This has necessarily led to the use of new descriptive terms, or to the redefining of old terms which should be spoken of in this place.

One of the findings of the present study is that there may be cardinal concentrations of oxygen for root-growth, and, further, that they

should always be related to definite temperatures; and also that they may be specific.

The least concentration of oxygen at which root-growth will take place is usually referred to as the minimum concentration. This expression is thrown out because it does not take into consideration the temperature of the reaction, but implies that the minimum may be a definite concentration, whether for a species or otherwise, and regardless of the temperature. The connotation is wrong. The least oxygen partial pressure for root-growth is here referred to as the *lower critical* concentration, or partial pressure of oxygen for growth, for the temperature to be given and for the species. In any species, as will appear later in this study, the lower critical concentration of oxygen is least at the low temperatures and greatest at the high temperatures.

A second cardinal concentration of oxygen for root-growth, which also is specific, is such as permits a "normal" rate of growth for any temperature. This concentration is called the *upper critical partial pressure*, or concentration of oxygen, and also must be related to temperature. At low temperatures the upper critical concentration of oxygen is least and at high temperatures it is greatest. The range in magnitude of both the lower and the upper critical concentrations of oxygen which accompany temperature changes may be small or large, as the case may be, inasmuch as the range of both appears to be a specific character.

The present study has mostly to do with root-growth in small concentrations of oxygen, but it also suggests something of the importance of studies on the upper critical concentration as well. The significance of the upper critical concentration to plant survival appears to be very great and possibly best ties the results of laboratory work with field observations.

An *oxygen deficiency* occurs when the concentration of oxygen for any species has values between the lower and the upper critical partial pressure. The amplitude of the deficiency of oxygen is apparently specific and is to be related to temperature.

Two additional cardinal concentrations of oxygen for root-growth should be recognized. It is known that growth may go on at a "normal" rate in widely different percentages of oxygen, but that when a certain concentration is exceeded, growth will decrease in rate. This may be termed the range of the optimum. At the lower limits is the upper critical partial pressure of oxygen. There is no reason to suppose that the range of the normal oxygen concentration for root-growth is not to be associated with temperature and with the species, although no work on this phase of the subject has apparently been done.

The final concentration of oxygen would be the true maximum beyond which no growth occurs at any temperature.

Reference has been made to "normal" conditions of growth. As it appears inconvenient always to avoid the term, it may be well to define it, as here used, somewhat exactly, although its intrinsic vagueness is acknowledged. However this may be, it has a certain convenience as a descriptive term. As used in this study, *normal aeration* is meant to imply a condition in which the oxygen-content of the soil approximates the partial pressure of oxygen supposed to obtain in the soil of the habitat of the species. When used in connection with a culture, it connotes a partial pressure of oxygen about equaling that of atmospheric air. And when a *normal growth-rate* is mentioned it means such a rate as would be expected to occur at parallel temperatures, in normal oxygen concentration, and at an equivalent stage of development.

It may be well to allude to the recognized circumstance that the "normal" may not be the optimal condition for a process, as for root-growth. Thus, for example, as regards growth-rate in roots, it appears to be true that at relatively high soil temperatures and in markedly aerobic species, as *Zea* and *Pisum*, 20 per cent oxygen constitutes a deficiency.

It will appear from the foregoing remarks on the relation of root-growth in an oxygen deficiency to the temperature of the substratum, that a deficiency of oxygen, as above defined, modifies the cardinal temperatures for growth as at present understood. Thus whatever, if any, may be the direct effect on the minimum temperature, the optimal and the maximal temperatures for root-growth are lower in an oxygen deficiency than in normal partial pressure of that gas.

Not only are the cardinal temperatures for growth of the root changed in a deficiency of oxygen, but other reactions also are modified to a greater or a less extent. Thus it is known that the capacity of a root to absorb water may be less in a small oxygen-supply than when the supply is normal, although the relation to temperature and to the amount of oxygen concentration appears not to have been determined. The matter also may be, and probably is, specific.

Again, it is known that the capacity of a root to react to the stimulus of gravity may be related to the supply of oxygen. Where this is inadequate the root may not exhibit geotropism. This, however, is possibly a relative matter also. It may be dependent in part on temperature and it is specific. Such as are markedly aerobic, as *Zea* and *Pisum*, for example, are incapable of reacting to gravity in a relatively marked deficiency of oxygen, whatever the temperature may be.

It is possible, also, that the development of root-hairs is directly associated with the partial pressure of oxygen. At what partial pressure of oxygen, however, or at what temperature root-hair formation will occur or cease has apparently not been learned. Moreover, the conditions under which root-hairs are formed may be unlike for dif-

ferent species, being possibly more critical in the case of marked aerobes than in the case of species not so dependent on a good supply of oxygen.

In intracellular processes and reactions, the dependence on oxygen is generally recognized. Not only is this the case in aerobic respiration, but also in many other processes and reactions associated with the formation, modification, or transfer of food materials and of foods, and their incorporation into the body of the plant. Thus the presence of oxygen is necessary in the removal of starch and possibly of oils from tubers, in the formation of cell-walls, in protoplasmic streaming. And it is of indirect importance to roots through its rôle in the formation of compounds containing nitrogen, as well as in other ways.

From the foregoing summary of certain physiological directions and means, the oxygen-content of the soil may operate to direct, or to modify, or to make possible a variety of processes in the root of vital importance to the root *per se* and through the root to the shoot; it is evident that other criteria than the growth-rate of roots might have been used to determine the direct influence of the oxygen of the soil on the physiology of the root. These, however, need not be taken up in this place.

In connection with possible specific variation of roots in relation to the reaction to oxygen, it appears that in the evolution of the organs of attachment of plants there have been not only numerous and complex morphological changes along many lines, but that the reaction capacities of roots, and of their functional equivalents, have also undergone developmental modifications which are manifest not only by differences in reaction to oxygen but in numerous other ways. Certain of these can be referred to briefly. In both high and low, simple and complex plants, physiological differentiation occurs early in the history of the individual through which the cellular derivatives of the zygote come to possess dissimilar qualities and unlike relations to each other, to the shoot, and to the environment. Thus the (fertilized) oospore has acquired characteristics apparently not possessed by, or which are masked in the (unfertilized) oosphere. The tropic reactions of the young plant are concerned not only with the internal environment, the mutual relation of tissues, organs, and especially of shoot and root, but also with the immediate external environment, of which the oxygen-content and temperature are important factors. It is of some interest to note that the organ of attachment is formed very early in the life-history of any plant, and that even in the lower plants it has forms of irritability which, as suggested above, are to a certain extent peculiar and which may not be shared by the shoot.

In the evolutionary development of plants certain reaction capacities of the primitive root, or its functional equivalent, have been lost, while the importance of others may possibly have been accentuated.

Such perhaps would be expected in view of the differences in the immediate relation to the external habitat of rhizoids, holdfasts, or roots, as compared to analogous relations of the shoot. For example, in the lower and submerged plants the external environment of shoot and of attaching organ is essentially the same. In the spermatophytes and to a certain extent in the higher vascular cryptogams, on the other hand, the portion of the oospore which becomes the root may never be in contact with certain features of the environment to which the shoot is more or less continually exposed. But, on the other hand, both shoot and root are subject to factors, like temperature and gravity and the like, which may or may not be quantitatively different for root and shoot. As to the oxygen relation, however, the partial pressure of oxygen to which shoot and root are exposed may be essentially alike, or, on the other hand, they may be widely different. But they are in agreement in the fact that under any condition the oxygen enters the tissues of the plant in solution.

The aeration of the substratum of the rhizoid, holdfast, or root is various and is influenced by many factors. Thus, in case of a water substratum, the oxygen-content is immediately affected by movements of whatever sort of the water itself, by the vegetation present, and through the latter by the character of the sky, to mention no other factors. Where the substratum is soil, its chemical and especially its physical nature importantly modify the possibility of its aeration. Of such the size of the soil particles has noteworthy and often striking effect. It is in soils of fine structure, as is recognized, that the conditions of aeration may be especially poor. Soils of this nature are especially likely to be puddled, in which case the carbon-dioxid content may become unusually high and the oxygen-content unusually low. Gaseous movements, in such case, are mainly by diffusion or in association with the movements of water. It is only upon drying out that molar gas-movement may take place and that a condition of aeration most favorable for root-growth occurs. Soils of open structure, on the other hand, may never be saturated. They have active gaseous exchange with the atmospheric air during all of the growing-seasons, and, at the depth attained by the roots of most plants, the amount of oxygen and of carbon dioxid remains about the same as in atmospheric air.

From what has been said relating to root-growth in a small amount of oxygen, and to temperature, it would be expected that a coincidence of oxygen deficiency and high temperature of the soil would limit root-growth, cut down water absorption by roots, induce wilting of the leaves, and possibly their fall. The root growth-oxygen relation thus becomes of possible importance in restricting the cultivation of economic plants and the distribution of plants in nature.

It has already appeared that there are two concentrations of oxygen,

in relation to root-growth, which are of especial moment, namely, the lower and the upper critical partial pressures. These two cardinal concentrations delimit the oxygen deficiency and like it are specific and intimately related to temperature. Although studies on species distribution from the standpoint of such oxygen relations have not been carried out, from the incidental findings of the present study the suggestion can be hazarded that limitations in distribution, where the oxygen relation is concerned, are to be associated rather with the upper critical concentration and its variation than with the lower critical concentration of oxygen. Species not tolerant of poor aeration conditions appear to have a high upper critical oxygen partial pressure for root-growth, while, on the other hand, this cardinal concentration is apparently low in species which are able to withstand poor conditions of soil aeration. It therefore follows that only species of the latter type are to be found where the aeration conditions are "poor." The converse, however, does not appear to be true. Both types of plants occur in loose soil, where the oxygen relation of roots probably does not play an important rôle in determining the nature of the flora.

In passing, a remark should be made on what, from the present point of view, would constitute "good" aeration of the soil. Owing to specific reaction of roots to oxygen and to the temperature relation, it is evident at the outset that no single term can be used to cover all cases; and, moreover, the definition should be with reference to the plant and not with exclusive reference to the soil; also, it is apparent that what may constitute adequate aeration for one species may not be so for another species. The relation, therefore, is complex to a high degree. However, from the standpoint of our present knowledge of the root-oxygen relation, it can be advanced that any soil is well aerated as to any temperature or species that permits a normal rate of root-growth. From this it will be seen that the oxygen-content, or the rate of oxygen delivery to the root, must at least equal that of the upper critical concentration of that gas. A well-aerated soil, therefore, is one which contains a partial pressure of oxygen equal to that of the upper critical concentration for any species. In some species this may be 2 per cent, or possibly less, but in other species 10 per cent and possibly 20 per cent oxygen. Thus, a water substratum or a saturated soil would be well aerated for certain species, while an open soil might not carry sufficient oxygen for others. The matter, therefore, lies wholly with the species, as will be apparent from the general relation of roots to oxygen and as already suggested in earlier paragraphs; and in this the temperature of the substratum plays a very important part.

In considering possible ecological application of aeration studies it is patent, from what has been said in earlier paragraphs, that the temperature-oxygen relation can not be separated. The work of

Howard shows this. Thus, in India, at the period of high soil temperature and also when the soil is saturated by monsoon rains, various direct effects on the physiology of cultivated plants are to be noted which have been attributed to poor soil aeration,[1] and which should be coupled to the fact of high temperature, as well. To quote from Howard:

"The almost complete cessation of growth during August and September coincides with the falling off of gaseous interchange between soil and air. At this period, the subsoil water has risen to its highest point, the surface of the soil has become exceedingly compacted by heavy falls of rain, cultivation is impossible, as the ground is too wet and the whole of the upper layers of soil have become waterlogged. There is no adequate air supply for the roots and exchange of gases between the air and soil is difficult. All growth tends to become arrested. Further, all vegetation at this time takes on a very unhealthy appearance. A change takes place as soon as the subsoil water falls and ventilation is possible. Active growth is resumed which goes on till the fall temperature brings about another resting phase."

Under similar conditions, as mentioned also by Howard, the direction of root-growth may be modified, and roots may be greatly injured, resulting even in their death, as reported by Balls for cotton in Egypt.[2] Under conditions of puddled soils, and also of high temperature, a portion of the roots of the cotton may be killed, and other ill effects may occur, possibly including the shedding of bolls in the cotton or of immature fruit in other species. Wilting of the shoot and permanent injury to the roots as results of known inadequate oxygen-supply are also given in the present study. It can be concluded, therefore that a deficiency of oxygen, together with high soil temperatures, supply important conditions to bring about a variety of physiological reactions inimical to the normal chain of processes in roots, and through roots in the plants as a whole. This, however, is apparently not true of all species, but only of those which are markedly aerobes. It has not been shown, for example, that such species as usually occur in soils, regularly saturated in summer, are injuriously affected by low oxygen-content of the soil. For example, the cultivated rice and species of *Salix*, not to mention other plants, appear to continue root-growth, and the shoots to remain turgid, in a soil atmosphere containing 0.5 per cent oxygen, provided the temperature of the soil is not high.

In this study emphasis has been placed on the reaction of roots in different percentages of oxygen when diluted with nitrogen. The gaseous mixtures mainly used, therefore, were different from atmospheric air only, or mostly, as regards the relative proportion of these gases. A certain number of experiments were carried out, however, with other gases as dilutants, among which was carbon dioxid. It has

[1] Howard and Howard. Soil ventilation. Pusa Agric. Res. Ins. Bull. 52, p. 11, 1915.
[2] W. L. Balls. The cotton plant in Egypt. Page 38, 1912.

long been known that this gas is not inert, but exercises recognizable effects on living organisms. In sufficient concentration it may bring about growth cessation, but in appropriate concentration it may temporarily accelerate the rate of root-growth. When used in high concentration together with atmospheric air the proportion of oxygen is cut down, which of itself may have a direct and added effect.

The quantity of carbon dioxid in the atmosphere of the soil is various, but usually it is not large. Thus, Russell summarizes the extreme limits of carbon-dioxid concentration in ordinary soils, that is, in arable lands, pasture lands, uncropped arable lands, sandy soil, grass lands, and the like, and gives 11.5 per cent as the highest found.[1] But this is much less than that determined in Indian soils by Leather, who reports having found about the roots of *Indigofera* as high as 21.14 per cent.[2] The concentration usually increases with depth, with the effect that although surface soils may contain approximately the same partial pressure as atmospheric air, at a depth of from 1.5 to 4 meters there may be as much as 1 per cent. It is doubtful whether the roots of land plants, even of those which may require exceptionally favorable conditions of aeration for growth, are influenced in any important or even determinable way by a soil atmosphere containing 1 per cent carbon dioxid, or even much more than that. Certain it is that often little, if any, direct effect on the rate of growth of roots, under exceptional conditions at least, is to be observed when the partial pressure of carbon dioxid is increased to 10 per cent. In higher concentrations, however, observable injury occurs; but there are specific differences as regards this, and, also, temperature may have an added effect. Thus, in certain species where growth ceased as a result of exposure to high percentages of carbon dioxid, its renewal occurred sooner at high than at low temperature of the soil.

Various means have been adopted by different experimenters to vary the oxygen-content of the substratum in studying the effects of variable aeration on plants. Among such the following should be mentioned: atmospheric air has been caused to stream through cultures; carbon dioxid, hydrogen, and to a certain extent nitrogen have been used to dilute oxygen; partial vacuums have also been employed. Adverse criticisms have not been wanting regarding the use of each of these, except only nitrogen. Further comment is not necessary in this place, except to remark that in case of many species, whatever the temperature, an increase in the available amount of oxygen, as by streaming atmospheric air, would not be expected to bring about an increase in the rate of root-growth. But in pronounced aerobes, as already remarked, with a high upper critical concentration of oxygen, such increase might take place, especially when the tem-

[1] E. J. Russell. Soil conditions and plant growth. Page 227, London, 1921.
[2] J. W. Leather. Soil gases. Mem. Dept. Agr. India, Chem. Ser. 4, p. 85.

perature of the soil is high, showing that the usual partial pressure of oxygen is in fact at such temperatures a deficiency. There have been used, in this study, in addition to nitrogen and carbon dioxid, argon and helium, and to a limited extent other gases as well, for the purpose of diluting oxygen. The possible toxicity of the gases is taken up in some detail in a special section of this report. It should be mentioned here, however, that, unless otherwise so stated, where percentages of oxygen are referred to, nitrogen is the diluting gas.

For convenience in the present investigation, volume percentages have been employed, although it is rather the rate of delivery of oxygen, for example, to the root than the partial pressure *per se* that matters. This was shown in various ways, including the effective differences of streaming gaseous mixtures, as opposed to static mixtures, in event of low percentages of oxygen. It also appeared when helium was substituted for nitrogen as a diluting gas.

Possibly enough has been given in the preceding paragraphs to indicate something of the complexity of the relation of root-growth to different conditions of soil aeration. It is evident that there is no means of telling *a priori* what the critical concentrations of oxygen for a species may be, but only by direct experimentation. The time has now come when such matters should be defined in more exact terms than have hitherto been used.

During the course of the investigations on root-growth in relation to the aeration of the soil, especial experiments were originated together with appropriate apparatus. Many hundred experiments were carried out and every important result was repeated until satisfactorily verified. Several representative species were used, although it is fully realized that the number of these should have been larger to allow safe generalization. The plants studied included seedlings, cuttings, and, to a limited extent, adults of herbaceous forms, shrubs, or trees. In every instance the species selected for study were such as either had previously been studied in the field or which were of economic importance. As to the latter, however, it is perhaps especially regrettable that each could not have been studied more closely. But the underlying idea of the work, which was to determine general facts of wide application rather than more minute facts, did not permit this. Intensive investigations of particular phases of the activities of roots were left to a later time.

As occurs in a long-continued series of studies along definite and planned lines, various researches of promise were suggested from time to time by the work as it progressed. But owing to limitations placed on the work it was not found possible, even when extremely desirable, to enter into most of these. Such physiological-ecological investigations, with especial reference to growth of roots, should be taken up, however, in the future. It is not necessary to present a list of the

suggested researches, although to do so would be a very interesting side light on the limitations of our knowledge of roots and of their relation to the shoot. They would also reveal something of the great economic importance of all studies on roots, and particularly those of a physiological-ecological nature. However, in addition to extending aeration studies to include more species, there are certain specific investigations and groups of investigations which merit early attention. Certain of these, relating mostly to growth of roots, can be referred to briefly in this place.

Investigations should be carried out on the mutual relations of root and of shoot, particularly with reference to the effect on root-growth. Relatively little or nothing is exactly known, for example, of the influence on growth of the root of varying the temperature and the light relations of the shoot, or of different factors associated with transpiration, or of the formation of leaves, flowers, or fruit; and, *per contra*, very little apparently has been done on activities of the shoot as affected by the root. The ecologic and economic importance and bearing of such investigations is apparent.

Especial and direct investigations on the respiration of roots in health and disease should be undertaken. With certain basal facts relative to root-growth in definite concentrations of oxygen and in relation to temperature now known, it should be possible to more profitably study gaseous exchange in roots. The investigation would be extended in a number of directions. For example, the present study suggests a possible day-night relation in growth of roots as associated with the absorption of oxygen. This should be followed out, and species which include those which are tolerant of poor soil aeration as well as those which require good aeration should be investigated. Downward translocation of oxygen from shoot to root, both as a possible fact and as a possible ecological factor of importance, should be studied in the same connection. Investigations should also be carried out on the respiration of roots of unlike types in association of shoots also of unlike types, as on fleshy roots of sclerophylls, fibrous roots of succulents, and on the root-shoot respiration of each type. What is now known relating to gaseous exchange in succulents makes such investigation the more important and the more promising of definite results.

Of additional and analogous researches on roots, in relation to growth, there is particular need of studies on the behavior of roots in reference to the presence of micro-organisms in the soil. Such studies would be of especial interest in connection with puddling of soils and consequent poor gaseous exchange and in connection with an especially low oxygen-content. The acidity-alkalinity of the soil in relation to growth of roots of different types while in soil is apparently also in need of investigation. Finally, mention should be made

of the great desirability of researches into various intracellular activities, mainly enzymatic, and intercellular activities, mainly diffusion, in association with growth of the root in soil.

The suggestions relative to desirable researches on roots are given not as an exhaustive list, but rather and only as work which urgently requires being carried on and with little delay. The investigations, in short, should comprise several phases of the physiology and ecology of roots, particularly those related to roots as reactive organs of plants, and being such as integral portions of the plant as a whole, indispensable to its well-being. Experimental studies on roots have been to a large extent incidental and not direct. They should be direct and purposeful. That such investigations are of fundamental importance, and that the results of researches on roots should be of wide application, both scientifically and economically, will be quickly recognized and needs no argument.

The writer wishes gratefully to acknowledge the frequent advisory assistance as well as continuous sympathetic interest which he has had for several years from Dr. E. E. Free, who also has collaborated to a certain extent in the work. Dr. Free has prepared an account, included in the present report, presenting the results of his studies on nitrogen and helium as inert gases.

APPARATUS AND METHODS.

In the experimental studies on roots, different methods have been employed from time to time and different kinds of apparatus, or none, have been used. Moreover, changes and refinements in both apparatus and methods have frequently been made as the work progressed, or revealed new leads, so that the procedure at present employed may bear but little resemblance to that earlier adopted. This has special reference to experimental studies carried on in the laboratory. Not all of the methods used need be described, nor all of the changes detailed, but an account of methods and apparatus most profitably employed at any time in the researches will be presented, and these, for reasons that are apparent, will be found to be sufficiently diverse.

Generally speaking, the introductory studies, which receive but scant attention here, or are not referred to at all, were exploratory, of a general nature, and did not necessarily yield conclusive results. They, however, were often suggestive and they pointed to problems or phases of the investigations which later were to be examined under more exact conditions.

In the more recent experimental work, of which the apparatus and methods will be first described, the aim has been to control as many of the factors as the facilities at hand permit, and at the same time it has been the plan to grow the plants under as nearly natural

conditions as practicable. Following such ideas, the plants have been grown in soil at known temperatures and as nearly as possible of constant or at least of sufficient water-content. The composition of the atmosphere of the soil cultures has also been under control and the apparatus has been so devised that gas analyses could be made when advisable. The methods devised and the apparatus employed in the studies on the differences between helium and nitrogen as inert gases in the experiments are described in another connection (p. 38). Those used in connection with the work on the effect of an excess amount of carbon dioxid or a deficiency of oxygen, where the diluting gas was nitrogen, are detailed in the present section.

The subaerial environment of the plants in the experiments has of necessity been largely neglected, although it is realized that it is impossible to wholly separate the factors which influence the shoot from those that affect the root, in part because of the close physical and physiological relation of the two, and in part because of the frequent reaction to a common environmental factor. When, however, the work shall be directed to an investigation concerned with the mutual relation of root and shoot, the influence of the one on the other, the subaerial environment must also needs be as guardedly under control as may be possible.

As presented in another connection, the plants studied have been fairly various; they are herbaceous, suffrutescent, or perennial. Although seedlings have been mainly used, cuttings also have been employed, as well as plants from tubers or bulbs. As actually experimented with, the plants have been from a few days to several months old, and the roots have varied from about 5 cm. to about 30 cm. in length. As a consequence, there is often a considerable period during which it is possible to observe the behavior of the root. For the most part, attention is confined to the main root, but whenever advisable the secondary roots also are observed. The use of the type of culture receptacles employed has made it possible, also, to study in some detail, when desirable, the finer points of the gross morphology of the root, as, for example, the formation of root-hairs.

It should be remarked in passing that, although up to the present time only the immediate effect of a variation in the aeration conditions of roots upon the rate of their growth has received attention at the hands of the writer, it has been obvious that such studies, when carried to a satisfactory conclusion, must include general work on the respiration of roots. For such investigations, as will appear presently, the method of control here described does not appear to be unsuited. It also may be appropriate for use in studies on closely connected features, as, for example, on root-growth and the moisture-content of the soil, on root-growth and the soil solution, including the alkalinity-acidity as shown by variations in the H-ion content, as well as on

the relation of the rate of root and shoot growth, and in other connections.

It will be the plan in the following running account of the methods and apparatus to describe the apparatus in some detail, so that, if desirable, it can be constructed and the experiments can be repeated. At the least a clearer view will be had as to how the results to be detailed were secured. It is not supposed that either the apparatus or the methods have any merit beyond that of achieving the ends they were planned to accomplish.

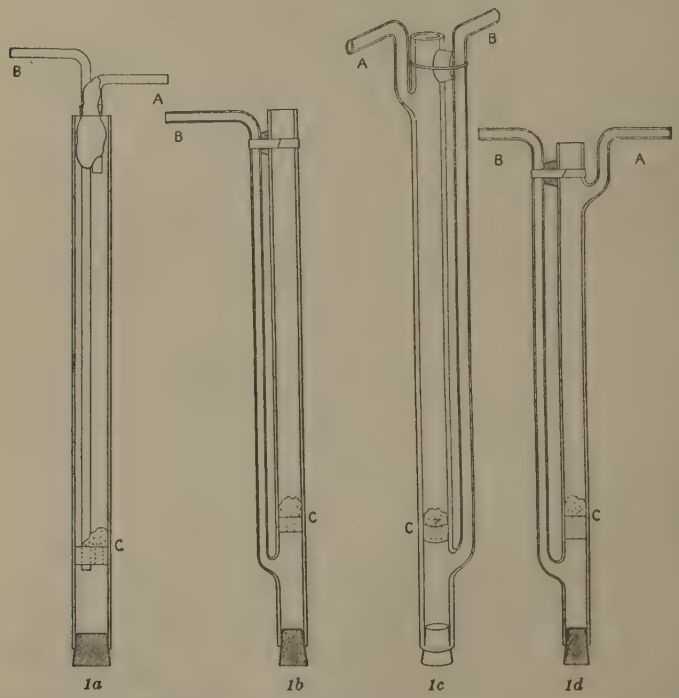

FIG. 1.—Different kinds of glass culture tubes used in studies on aeration relations of roots. *A* and *B*, inlet and outlet tubes, of which the former does not appear in 1*b*; *C*, perforated cock septum with cotton or glass wool. Inlet and outlet tubes in 1*a* and 1*b* are attached to the inside of the larger tube with sealing-wax or de Khotinsky cement.

The plants to be studied are grown in glass tubes, care being taken to select a kind of glass that will not easily break. The culture tubes are 45 cm. long and 2.5 cm. inside diameter. They are of several types, but agree in this, that there is an inlet and an outlet tube suitably placed for the conduction of the experimental atmospheres. The different kinds of tubes mostly used are shown in figure 1.

In the types of tube illustrated by figure 1*a*, both intake and outlet tubes are placed on the inside of the larger culture tube, to which they are firmly attached by de Khotinsky cement or sealing-wax. The longer of the two goes downward to a perforated cork septum, through which it passes, which is situated somewhat above the lower end of the tube in the manner indicated in the figure.

In the second form of culture tube the outlet tube is attached to the larger tube not far above the lower end and is continued upward to the upper end of this tube, where it bends at right angles to it, either in the same plane as the two parallel tubes, or at right angles to them. This type is shown in figure 1*b*. In this tube the inlet tube is short, bent at right angles, and attached to the inner surface of the culture tube as in the case of the similar tube of the first type. It is not shown in the figure.

The third type of culture tube has both the inlet and the outlet tubes attached as indicated in figure 1*d*. In this case the culture tube is somewhat smaller than the form usually used, as it was constructed for the purpose of observing root-growth under aseptic conditions and its size was limited by that of the sterilizer in which it was placed.

The fourth type of culture tube, figure 1*c*, is of the standard dimensions and has both outlet and inlet tubes as indicated by the figure. In this case the final bend of each tube is at right angles with the plane formed by the parallel tubes, but the two take opposite directions, so that when the culture tube is in place in the thermostat the outlet of the outlet tube is on the lower side of the culture tube, and the inlet tube opens on the upper, opposite side, as indicated in the figure.

A cork septum with perforations is placed about 10 cm. above the lower end of the culture tube, in the first type described, and somewhat above the attachment of the outlet tube in the others. A plug of glass wool or of cotton rests on the septum and prevents the downward escape of soil, but does not prevent the passage of gases or of water. The lower end of the culture tube is closed by a rubber stopper.

The free upper end of the outlet tube is stayed by means of a piece of cork wedged between it and the main tube and further anchored by wire, or preferably by a few windings of electric tape in the manner shown in the figures.

An additional type of tube was devised for use when weighing was desirable. This was similar to the tube shown in figure 1*d*, except that a ground-glass stopper of light weight was fitted into the lower end in place of the heavier rubber of the other tubes, and that the walls were as thin as was consistent with the load the tube was used to carry. This type of tube weighs, when empty, about 122 grams as opposed to about 189 grams of the type commonly used. The dry weight of soil, sand, required to load the culture tubes is about 207 grams.

The position of the cork septum, in the first type of culture tube described, or of the attachment of the inlet tube of the other forms, is about 10 cm. above the lower end of the culture tube. Thus a sump of considerable capacity is formed to receive drainage of excess water from the soil above, to the end that the lower end of the outlet tube is not blocked by water, and, moreover, it can be made to store sufficient water so as to irrigate the culture by up-ending, if desirable. In certain experiments, also, where it was necessary to introduce absorptive material into the culture tube, the sump was a convenient place for this purpose. Thus, where it was desired to absorb carbon dioxid as it was evolved from the root of the plant, a small vial containing potassium hydroxide was introduced and kept in the sump throughout the experiment. Also, in experiments in which it was necessary to remove all traces of oxygen, untarnished iron wool, which was kept moist, was left in the sump. Any traces of oxygen which may have escaped the previous treatment of the gas, or which may have diffused into the experimental gaseous mixture, would be taken up by the iron wool.

The gas capacity of the culture tubes with inlet and outlet tubes, including the pore space of the sand, was determined approximately by replacing the air of the tube with water. This was found to be about 100 c. c., which is many times the volume of the primary root or of the entire root system of young seedlings.

After the plant to be studied has attained sufficient size for use and is growing in the culture tube, it is sealed in by the use of plasticine, grafting-wax, cocoa-butter, vaseline, or paraffin, or with combinations of these. In bulbous plants, or in species having leaves arising from a short axis, it has been found necessary to adopt especial precaution against the inward leaking or diffusion of atmospheric air. In such cases vaseline, grafting-wax, or cocoa-butter is used to coat the portion of the plant through which the leakage is suspected to take place before plasticine is used.

The soil used in all experiments is sand from a wash on the grounds of the Desert Laboratory, Tucson. The sand was passed through a 20-mesh (inch) sieve. The sand is commonly used unsterilized, although in certain uncompleted experiments sterilization was attempted.

When thoroughly moistened, the sand in a culture tube is sufficiently moist for three or more days, depending on the extent of the absorbing surface of the root among other factors. As mentioned elsewhere, at the beginning of an experiment the sand is thoroughly saturated with water by drawing it through by gentle suction. Under such conditions there is left about 17 per cent, dry weight, of the soil, of moisture. The water-holding capacity, however, of the sand is approximately 23 per cent dry weight.

If during an experiment it is desired to irrigate the culture without opening it, this can be easily accomplished by up-ending the tube and making use of the water held in the sump as a reservoir, as mentioned above.

Constant temperature of the cultures is attained by conducting the experiments in a room the temperature of which is constant, or in a chamber of appropriate size the temperature of which is controlled, or by the use of root thermostats especially devised for the purpose. Under the conditions first mentioned, the shoot and the root are under the same temperature conditions, but in the last instance that of the root only; that is, the culture tube is maintained at constant temperature, and the temperature of the shoot varies with that of the room where the experiments are carried on.

To effect the control of the temperature, electric regulators of different types have been employed. These have been bimetallic, mercurial, or of gas and mercury combined. In the earlier studies a gas-mercury regulator, which was extremely sensitive to temperature changes, was used. This type was U-formed, with a portion of one arm expanded into a bulb. Mercury filled the lower bend up to 1 cm. of the bulb, above which a movable copper contact was tightly sealed, with the end of the wire adjusted to touch the mercury when the proper temperature was reached. A contact wire dipped permanently into the mercury of the other arm of the U. Proper connections with a series of dry cells, and with a relay which operated a switch by which the heating current was turned on or off, were made. In use the glass U-thermo-regulator was immersed to the upper portion of the bulb in the water of the thermostat. With the movable contact just touching the mercury at the desired temperature, additional heating caused expansion of the gases in the bulb which brought about a downward movement of the mercury below the bulb and with it the breaking of the battery circuit, and, eventually, that of the heating circuit. It will be observed that in this type of thermo-regulator the action is opposed to that of the so-called thermometer type, in that contact is made only when the thermostat is too cool and not when it is too hot. This is regarded as a factor making toward safety.

Two all-metal thermo-regulators, commercial types, were mainly employed. Both were used without relay, but were connected directly with the heating circuit. Although not so sensitive to temperature changes as the glass-mercury regulator above described, they have been found sufficiently so for the purposes in hand. The type shown in figure 2 is mounted on a wood base and kept tightly inclosed in a water-tight metal box which is deeply immersed in the water of the thermostat. Removal from this metal receiver is necessary for regulating. The second form of thermo-regulator employed, not illustrated

in the figures, is also bimetallic and operates on much the same principle as the one last referred to. Further description is not necessary. By the proper manipulation of either of the all-metal regulators, the water in the thermostat can be kept within about 0.5° C. in a range from about 20° to more than 35° C.

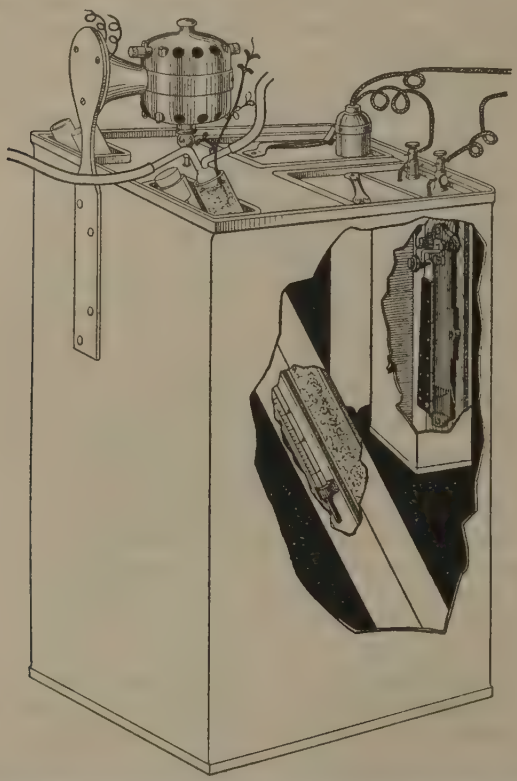

FIG. 2.—Root thermostat with portion of side removed to show construction and relation of parts. Dimensions are given in text. In practice, the liquid-stirrer is attached to a wooden support apart from the thermostat itself, but here shown otherwise for convenience. One type of thermo-regulator formerly used is shown in place. The plant studied is shown in a culture tube, in the same compartment with which is a tube with thermometer. Tube compartment closed at upper end by cotton wool (not shown).

The heating elements employed are electric bulbs of from about 125-watt to about 250-watt capacity.

A special form of thermostat was devised for use when it was desirable to control the temperature of the root. In such case, as previously mentioned, the temperature of the shoot followed that of the room, or chamber, where the experiments were carried on.

In some instances the temperature of the room or chamber was constant, but for the most part it was not.

A root thermostat has capacity of carrying from 2 to 6 culture tubes, depending on the form of tube used. A battery of from 4 to 6 thermostats is employed simultaneously in any experiment.

The root thermostat as used is a receptable made of galvanized iron of good weight and measures 28 by 28 by 44 cm. (fig. 2). It is water-tight. Four rectangular openings are left in the upper end, of which two are 10.5 by 14 cm. and are provided with inserted covers. The other two rectangular openings, 4.5 by 9 cm. in size, are not closed with covers, but are designed to carry each a long and squat carrier for the culture tubes. The tube carriers are 3 by 8 by 50 cm. and are also made of heavy galvanized iron and are water-tight. Although made to fit snugly, the carriers can be easily removed from the thermostat. Their inclined position when in place is shown in figure 2. The two larger covers are provided with openings for the insertion of whatever form of heating element and thermo-regulator may be used, which, as indicated by figure 2, project well into the thermostat.

In addition to the foregoing, a liquid-stirrer is provided and so placed that the lower end of the revolving shaft also projects deeply into the thermostat. In figure 2 the artist has represented the stirrer as attached to the thermostat. In actual practice, however, it is fastened to a substantial wooden frame immediately back of the thermostat and the shaft either projects vertically downward through the top as shown, or an opening is made at the extreme upper end of the back side, through which it is run at an acute angle. In any event, when the thermostat is filled nearly to the top with water, the stirrer shaft, as well as the heating element and the thermo-regulator, extend some distance below its upper surface.

A circuit-breaking clock, not shown in figure 2, is employed to interrupt at regular intervals the current which actuates the stirrers. For the purpose in hand, it has been found satisfactory to allow the stirrers to operate for a period of 1 minute, 3 or 4 times an hour. This appears to prevent noticeable stratification of the temperature of the water, with the effect that the cultures are maintained at practically the same temperature throughout their length.

When in place the culture tubes project little, if any, above the tube-carriers, although both the inlet and the outlet tubes are sufficiently long to well clear the upper end of the carriers, allowing room for necessary manipulation. A control glass tube, similar to the culture tubes, or a tube carrying a thermometer, can, if desired, be placed in the tube carriers along with the culture tubes. Finally, cotton is snugly packed about the upper ends of the culture tubes for the purpose of insulating them, thus largely preventing changes of temperature of the tubes from external causes.

In case the thermostats are so situated as to be exposed to the temperature changes of the room where the experiments are being carried on, they are inclosed in some insulating material, as heavy building or carpet paper. A satisfactory practice has been found, however, to immerse them nearly to the top in soil, keeping each thermostat separated from its neighbor by 5 cm., more or less.

When properly working, the thermostat maintains the temperature of the cultures within a variation of about 0.5° C., or even less, which is sufficiently accurate for the nature of the experiments with which they are used.

For convenience in transportation as well as for convenience in setting up in permanent or in temporary quarters, the thermostats have also been permanently built into cases of appropriate size, 3 in each case. Thus, two banks of thermostats are the result of this arrangement when 6 are in use, giving as many possible temperatures. Where this has been done it has been found advisable to have condensers of suitable capacity for each regulator, as well as for the circuit-breaking clock, all of which, together with the wash-bottle, the purpose of which will be given below, securely attached to the case, and appropriate resistance for each stirrer is arranged in such fashion that any stirrer can at any time be cut out, or caused to revolve more or less rapidly, according to the amount of resistance included in the stirrer circuit. It has been found possible also to include gas-piping with metal cocks as a permanent feature of each case. Finally, the two banks of thermostats are joined together, when in use, by an appropriate plugging-in of corresponding electric systems, and by a joining of the gas-pipes through rubber tubing. When the two banks are so united the one circuit-breaking clock and the one wash-bottle is sufficient for all. It should be added that each thermostat is insulated by heavy paper.

The gases employed are commercial nitrogen, oxygen, and carbon dioxid stored in cylinders under pressure; in addition to these gases, argon and helium, especially procured, were used in certain experiments. As received the gases are not pure. The nitrogen contains about 0.5 per cent oxygen, the helium about 0.2 per cent oxygen, and the other gases somewhat larger amounts. As detailed in another part of this study, examination of the nitrogen and helium used showed that they did not contain substances deleterious to plant or animal life, and that possible harmful effects resulting from their use could come only from the almost total absence of oxygen.

Metal and glass gasometers are used for mixing the gases and for storage during experiments. The gasometers are of two capacities, namely, 20 and 40 liters, and are so arranged that by the employment of weights slight pressure is put on the confined experimental gas mixtures. In both types of gasometers the gases are stored over

water, although heavy paraffin oil is sometimes used to lessen the inward diffusion of oxygen from the atmospheric air.

Between the gasometers and the culture tubes, a wash-bottle and glass distributing tubes are inserted in the gas line. The former serves the double purpose of revealing leaks in the culture tubes, which, if present, are always outward, and also of keeping the gas mixture moistened. Each distributing tube carries 2 or 4 outlets, which are connected by heavy rubber tubing to the inlet tube of each culture tube. All of the unions are of similar rubber tubing. Whenever the distance between the gasometer and the thermostat being used is relatively great, long glass tubes take the place of rubber, which is used only for unions. Finally, it should be mentioned that the outlet tube is provided with a short rubber tube, carrying a short piece of glass tubing. All connections are securely wired and when advisable are coated with shellac. Metal pinchcocks close all openings and are frequently attached to unions for the purpose of isolating individual cultures for whatever reason, as when gas analyses are being made.

In the preceding paragraphs a sketch has been given of the leading points of the apparatus mostly used in connection with the aeration studies on roots. In addition to the apparatus described, and varying from it little or much as the case may be, other means were employed in the general investigation. These will be described below in this section, or later in the running accounts of such experiments as may have been based on them. It will be advisable to now give the leading features of representative experiments in which the apparatus just described was employed.

On August 10, for example, an experiment was set up in which there were 10 cultures of Golden Bantam corn seedlings, in tubes of the type shown in figure 1*c*. Two cultures were placed in the thermostat running at 30°, and two each in thermostats at 27°, 22°, 21°, and 18° C. Each seedling was sealed with plasticine about the crown of the main root, and the seal included the base of the leaves as well. As the purpose of this description is merely to show the method employed in the experiment, it will be necessary only to trace the course of one of the cultures, and for this purpose that of No. 1 will be suitable.

At the beginning of the experiment the main root of No. 1, the only root in view against the concave lower and inner surface of the culture tube, was determined to be 15.5 cm. long. Just previous to the experiment the culture tube was given Knop's normal nutrient solution, although occasionally tap-water is also used, and the excess drawn gently through the soil, so that there was little or no capillary water remaining, and the pore space was relatively great. Culture No. 1 was then put in the 30° C. thermostat and cotton packed about

the upper end of the tube. In this position, as indicated in figure 2, the shoot and the outlet and inlet tubes of the culture tube only projected out of the tube container of the thermostat.

The position of the tip of the main root was marked on a gummed paper attached to the culture tube, and after the culture had been in the thermostat 24 hours, on August 11, the position of the root-tip was again marked. During this time the root had increased 45 mm. in length. Both the inlet and the outlet tubes had remained open and the root was assumed to have had "normal" aeration.

On August 11 the culture was sealed and connected with the gasometer containing nitrogen with sufficient oxygen to make the latter 3.6 per cent, volume, of the whole. All connections were secured by wiring. The outlet tube was fastened with a screw pinchcock, and it was seen that although the gaseous mixture in the gasometer was under some pressure, there was no outward gas leakage in the culture which would have been indicated at once by the wash-bottle in the gas train. The pinchcock on the outlet tube was unfastened and gentle suction was applied by which the experimental gaseous mixture was drawn slowly into and through the moist sand. This was maintained until an amount of the experimental atmosphere equal to approximately 10 times the volume of the culture tube and inlet and outlet tubes had passed through. Attachment was then made to a standard commercial gas-analyzing apparatus and the amount of oxygen present in the culture tube was found to be 3.6 per cent, or the same as in the gasometer. The outlet tube was then closed and the culture left for 24 hours.

On August 12, after having thus been in the gaseous mixture one day, the position of the root-tip was marked and it was found that the growth of the root in the mixture and for 24 hours had been 19 mm. The amount of oxygen in the experimental atmosphere was again determined by drawing through the culture tube in lots of 50 c. c. each, and it was found to be 3.6 per cent.

The culture was thoroughly aerated on August 12, but not irrigated, and both outlet and inlet tubes were left open.

On August 13, the position of the root-tip was again determined and it was found that during the preceding 24 hours, while in "normal" soil atmosphere, a growth of 42 mm. had taken place. The culture was then irrigated as at the beginning of the experiment and a gaseous mixture containing 10 per cent oxygen was administered.

The position of the root-tip on August 14, after the root had been 24 hours in 10 per cent oxygen, was marked, and it was seen that a growth of 37.5 mm. had taken place.

The culture was aerated August 14 and was allowed to remain a final 24 hours in the 30° thermostat, at the expiration of which time, August 15, a growth of 40 mm. was found to have occurred.

On August 13, when 10 per cent oxygen was given, it was not considered necessary to make a determination of the oxygen, for the reason that by an arrangement on the gasometer, and with knowledge of the oxygen-content of the nitrogen used, it is possible to compound an experimental atmosphere containing oxygen within an error of 0.5 per cent of that gas with fair certainty. In all instances, however, where a small quantity of oxygen is concerned, 3 per cent or less, oxygen determinations of each culture are made with alkaline pyrogallol at the beginning of each experiment, at the time of change of experimental atmospheres and, in long experiments, frequently daily.

The quantitative determination of the amount of oxygen is accurate to about 0.2 per cent volume, and possibly less. In practice the oxygen analysis of the individual culture is made as follows: Attachment to the apparatus (Orsat) is made with as short tubing as practicable, and the first 50 c. c. drawn off is discarded. This is about the capacity of the outlet portion of the culture tube. Following this the gaseous mixture is analyzed in successive 50 c. c. quantities until the readings are found to be the same, which will agree with that of the stored mixture in the gasometer. Occasionally a blank tube with moist sand but without a plant is used as a check. In one such the following percentages of oxygen were determined, that in the gasometer being about 1 per cent. The first 50 c. c. was discarded, and following this the successive determinations were 0.8, 1, 1, 1, and 0.9 or 1 per cent, respectively, which is representative of the oxygen variation of blank controls.

The analyses of tubes carrying living plants were not so uniform as those in which only sand, with micro-organisms, were present. For example, on June 1 the determination of the oxygen-content of the soil atmosphere of an onion culture was made. There was 1.2 per cent oxygen in the storage tank. After 50 c. c. had been drawn off from the culture and discarded, the following percentages of oxygen were found in successive analyses of 50 c. c. of the gaseous mixture, namely, 1.8, 1.6, 1.4, and 1.8, and after the removal of several hundred cubic centimeters the amount was found to be 1.2 per cent, or that of the mixture in the gasometer. In this instance it was found, as will be remarked later, that there was inward gaseous diffusion from the atmospheric air, a fact only to be detected by oxygen determination of the individual culture.

Although there is danger of inward diffusion of oxygen under almost any condition, it appears to be relatively little or so small as not to be determined by the method used in many instances. Thus, a culture of *Prosopis* was given an experimental atmosphere containing 0.6 per cent oxygen June 14. After having been 24 hours in this mixture, the amount of oxygen present in the culture was determined to be 0.6 per cent, indicating that the amount consumed by the small

root system could not be detected by the method, or that inward flow or diffusion was sufficient to counterbalance this. If, on the other hand, the root system is relatively extensive, there may be measurable depletion of the amount of oxygen in the soil atmosphere. For example, on May 29 a culture of *Pisum* was given 1.6 per cent oxygen for 24 hours. At the end of this time the following oxygen determinations for the culture were made: The first 50 c. c. was discarded; following this three successive determinations of 50 c. c. lots gave 0.6, 0.8, and 1.6 per cent oxygen. In another culture run at the same time and treated in all regards the same, no root-growth occurred, and in this the successive determinations of oxygen were 1.8, 1.6, 1.4, and 1.6 per cent, indicating possibly little or no absorption on the part of the root, but, on the other hand, possibly slight inward diffusion. A wholly analogous result was had on June 2 with another *Pisum* culture, where root-growth was found to cease in 1.6 per cent oxygen at the temperature used. In this the successive oxygen determinations gave 2.2, 2, and 1.8 per cent oxygen, with 1.6 per cent after a larger quantity of the mixture had been withdrawn. The last reading was the oxygen concentration in the gasometer.

Oxygen determinations of blank controls, of which one has been given, indicate that the variations in the oxygen-content are not wholly due to inward diffusion through the seal, or through rubber connections, but that they are at least in part due to the roots themselves, or at least to the presence of the living plant. It would possibly be helpful in determining the oxygen requirement of the subterranean parts if the mass of the living material were known as well as the area of the surface capable of absorbing oxygen. There are evident difficulties in the way of measuring with a useful degree of accuracy either of these features. That root systems of seedlings vary widely in the extent of the roots, however, is a matter of general observation. Two illustrations can be referred to. A certain seedling orange which was studied, and which was sufficiently developed so as to have small but mature type of leaves, had a root system of which the main root was 10.1 cm. in length. There were 13 laterals from 0.5 to 1.5 cm. long. As determined by replacement, the entire root system had a volume of about 0.2 c. c. Owing to the absence of root-hairs, the entire absorbing surface is extremely meager. At the other extreme can be mentioned a seedling cotton, fairly old, whose roots were among the most extensive of any plant examined. The specimen referred to had a main root 18 cm. long and 40 laterals, varying from about 1 to about 5 cm. in length. The moistened root system replaced about 0.8 c. c. of water. Root-hairs were abundant, so that the difference between these two plants, so far as the absorption surface is concerned, was much greater than the difference in bulk would lead one to suppose. The presence of root-hairs increases the surface area

10 or 12 times. Although in the two instances the surface area is not known, it can be seen from the great difference in bulk of the roots, as well as from the absence in the one case and the presence in the other of root-hairs, that the difference in the area devoted to absorption is very great indeed. There is also, without doubt, a very great difference in the amount of living tissue, as indicated by the difference in volume of the two root systems, and with this a corresponding difference in the demand for oxygen. That this is the case was suggested by the oxygen determinations of cultures of the two plants. Of these it need only be mentioned in this place that the root of the cotton plants, when given 1.2 per cent oxygen, stopped growth, and that analyses of the oxygen-content of the culture, after having been 24 hours in a gaseous mixture with 1.2 per cent oxygen, gave for the second 50 c. c. only 0.8 per cent of that gas, but the expected amount was determined in later readings.

It might appear in such instances as the last that the amount of oxygen present may be insufficient for the needs of so extensive a root system, and this, indeed, may be the case. It should be pointed out, however, that the amount of oxygen actually within the culture tube was relatively large, for the reason that although it was only 1.2 per cent of the atmosphere of the soil, the amount was approximately 125 times the bulk of the roots, whatever significance this may have, so that the amount of living tissue was relatively small. It seems rather that the rate of delivery of oxygen to the absorbing surface of the root may have been insufficient for the needs of the root, and not the partial pressure of the gas of itself. This can be illustrated by referring to a single experiment out of many in which the principle was demonstrated.

On September 30, two *Pisum* cultures were given helium in which there was 0.2 per cent oxygen. In one of the cultures the gaseous mixture was static, but in the other it was caused to pass very slowly through the soil. At the expiration of 48 hours the root in static atmosphere was seen to have increased 7.5 mm. in the experimental atmosphere, while that in the mixture which was slowly streaming grew 10.5 mm. In another example, 0.5 per cent oxygen with nitrogen was administered both static and streaming. In the former culture root-growth promptly ceased and none was recorded during 24 hours, but in the latter culture, with the slowly streaming mixture, the root grew 12 mm. during this time. During the following 24 hours, however, when the latter was given the experimental atmosphere statically, growth ceased.

Such experiences, and those above referred to, appear to indicate that the culture tubes have sufficient volume not only to afford a certain amount of normal root development, but to normally contain sufficient oxygen to make this possible. Further than this, it is

apparent that even when a small percentage of oxygen is employed, the volume of the culture tube is sufficient to permit the inclusion of so large an amount of the experimental atmosphere that the effects noted should probably be attributable to the small proportion of oxygen present in this atmosphere, which amounts to the rate of delivery, rather than to there not being sufficient oxygen in the culture tube because of its limited size. In other words, with an equal percentage of oxygen the same results would be expected to occur, no matter how extensive the subterranean gaseous reservoir.

In the preceding paragraphs on methods, emphasis has been placed on the necessity of frequently determining the amount of oxygen present in the culture tube, and hence available to the roots in the soil therein. There is also an additional factor of the very greatest importance to which close attention must also be given, namely, the temperature of the soil. This is based on the well-recognized increase in the demand for oxygen which accompanies an increase in temperature. According to Pfeffer,[1] respiration may be "twenty to forty times more active at the maximum temperature (for growth) than it is at 0° C., and the activity frequently increases in geometric proportion as the temperature rises to the maximum, while the respiratory quotient $CO_2 : O_2$ usually, but not always, remains constant." That the respiratory ratio has been found to change with temperature variation[2] does not greatly alter the case.

The temperature relation can be illustrated by referring to observations on the growth of the root of *Pisum* in a soil atmosphere containing 1.6 per cent oxygen and with the culture at the following temperatures, namely, 17.5°, 27°, and 30° C. At a soil temperature of 27° and in "normal" soil atmosphere, the root increased 31 mm. in 24 hours. During the second 24-hour period the root was kept continuously in gaseous mixture containing 1.6 per cent oxygen, with the temperature maintained at 27°, and the growth fell to 2 mm.; this probably occurred during the first part of the period. The culture was then transferred to a thermostat running at 17.5° and was aerated. The root-growth during the following 24 hours was 5 mm. Thereupon 1.6 per cent oxygen was again given for 24 hours, and the growth was seen to be 3 mm. at a soil temperature of 17.5°. At the conclusion of this test the culture was placed in a thermostat which was kept at 30° and was given 1.6 per cent oxygen. During the following 24-hour period no root-growth occurred.

It is apparent from the foregoing that the cardinal temperatures for root-growth may be changed by greatly diminishing the supply of oxygen, which is of interest in view of the fact, as given by Palladin, that the value of the respiratory ratio is not changed under such

[1] Physiology of plants, vol. I, p. 562. Eng. trans.
[2] Paladin's plant physiology, p. 191. Eng. trans.

circumstances. In the case of seedlings of a variety of *Zea* (Golden Bantam corn) the optimum temperature for shoot-growth is less than 35°, which is apparently not greatly below the maximum for root-growth in the variety, and the optimum temperature is about 30° C. This presupposes an adequate oxygen-supply. In about 3.6 per cent oxygen, for instance, although the rate at 30° is greatly below normal, it may be the most rapid for that partial pressure of oxygen, but when the amount of oxygen is reduced to 3 per cent, or under, the most rapid rate of root-growth appears to go on several degrees below 30°, or, in other words, the optimum temperature is greatly lowered. In a diminished supply of oxygen, as will be spoken of again somewhat fully, the maximum rate is possibly always lowered and to a degree directly related to the partial pressure of that gas. So far as a minimum temperature is concerned, it appears possible that the diminished supply of oxygen may operate, under certain conditions, to raise it above that normal for the usual condition of aeration. These conditions assume a fairly high minimum for root-growth. Where, however, the opposite is the case, the effect is questionable. The establishment of the cardinal temperatures for the growth of roots is clearly an important antecedent to studies on the relation of root-growth to a diminution in the oxygen-supply, just as a record of the soil temperature during the course of the experiments is also essentially necessary.

In experiments relating to the effect on root-growth of a diminished supply of oxygen, the amount of oxygen present must be known with a fair degree of exactness, that is, within 0.5 per cent, or preferably less. Reference to the rate of root-growth in seedlings of *Prosopis* will be sufficient to illustrate the point. At a soil temperature of 20°, the rate of growth is about one-seventh normal in 0.6 per cent oxygen and about one-third normal in about 1 per cent oxygen. In about 2 per cent it is about 0.7 normal. Where the oxygen pressure is so low, therefore, the rate of growth of the root of *Prosopis* is approximately proportional to the amount of oxygen available.

A comparison of the rate of root-growth of different species at parallel temperatures and in similar but varying amounts of oxygen constitutes a useful means of contrasting different species as to their oxygen relations. A single illustration will be sufficient in this place. At 27° C., the root of *Allium cepa* has a rate of growth about eight-tenths as rapid in 3 per cent oxygen as in normal soil atmosphere, while at the same temperature and in the same amount of oxygen the rate for *Zea* roots is about 0.1 as rapid as under normal aeration conditions.

The general method above outlined was mainly used in the studies on the relation of root-growth to qualitative variations in the atmosphere of the soil, but in addition to this another method was employed to a small extent. By this one the same culture tubes were used and

the same manner of handling the gases, but cultures were not kept in thermostats and the observation periods were short. Measurements were made with a horizontal microscope which was kept focussed on the root constantly. By this direct-observation method, slight movement of the root-tip could be detected and measured, and the reaction-time could be learned in a manner impossible by the method most employed. Although not suitable for long-continued experiments, and of limited use because of the need of a microscope for each culture, it nevertheless has proved to be a convenient means of demonstrating root-growth under somewhat varied experimental conditions.

In the direct-observation method, both culture tube and microscope are permanently attached to a small platform, with a floor inclined about 20° from the horizontal, and so arranged that it can be moved from place to place as desired without disturbing the cultures. A thermometer is attached to the culture tube. The entire apparatus can be kept in a constant-temperature chamber or room, or the actual temperature of the soil can be recorded for each observation in case the laboratory has fluctuating temperature.

Illustrations of the method will indicate something of its usefulness and also its limitations.

On November 23, a gaseous mixture consisting of 1.5 per cent oxygen, with the balance nitrogen, was given three seedling peas. The observation periods, growth, and temperature of the air near the cultures at the time of each observation are given in table 1.

TABLE 1.—*Root-growth of pea in 1.5 per cent oxygen, with nitrogen.*

Growth periods.	Growth.	Temp.
	mm.	° *C.*
$11^h\ 45^m$ to $12^h\ 15^m$	0.3	22
12 15 2 15	.5	20.5
2 15 3 15	.3	19
3 15 4 15	.2	16.5

Root-growth was observed to occur before the administration of the experimental atmosphere, but it was not measured, making it

TABLE 2.—*Root-growth in pea in 1.1 per cent oxygen, with helium.*

Growth periods.	Growth.	Temp.
	mm.	° *C.*
$11^h\ 50^m$ to $2^h\ 00^m$	[1]2.5	20.5 to 22.5
3 40 4 30	.3	20.5 18.5
4 30 8 40 (a. m.)	2.3	18.5 8.5

[1] Root in normal soil atmosphere.

impossible to indicate the decrease in rate which was attributable to the reduction in oxygen. In the following experiment (table 2)

in which 1.1 per cent oxygen was used with helium, the growth of the root before and during the use of the gaseous mixture was recorded.

The first of the two experiments quoted above demonstrated the fact that in the variety of pea used, root-growth will continue for a short time, in this case over 4 hours, in a soil atmosphere containing only 1.5 per cent oxygen, balance nitrogen, which was confirmed by the other method, and the second of the experiments showed that if the diluting gas is helium, growth will continue in *Pisum* even in 1.1 per cent oxygen.

The direct method was found somewhat more useful in the observation of the immediate effects on the rate of root-growth of an excess of carbon dioxid in the atmosphere of the soil. To detect the momentary stimulating effect of the mixture, some such method, indeed, seems to be required. The following will be sufficient to illustrate the point. In studies on *Prosopis velutina*, one part atmospheric air was mixed with 3 parts cylinder carbon dioxid and the mixture was given the roots continuously for about five half-hour periods. The rate of growth was observed also during a short time just preceding the gas administration. At the beginning of the observations the temperature of the air was 26° and at the close it was 22° C. In normal soil-air the average growth-rate of the root for half-hour intervals and at the observed temperature was seen to be 0.55 mm. With the administration of the gaseous mixture, however, the following half-hourly growth increments were recorded, namely, 0.66, 0.52, 0.19, 0.26, and 0 mm. The momentary stimulating effect of the mixture, rich in carbon dioxid, and the final inhibition of growth are clearly indicated. It would have been possible also, although it was not done in the experiment referred to, to observe the length of time elapsing after the replacing of the experimental atmosphere with atmospheric air, before a normal growth-rate would occur. In certain species, as will be detailed in another place, the recovery time is apparently specific and thus may not be the same for all plants.

Methods in which the apparatus above described were, at least in part, used and yet other methods with quite different apparatus, or with none, were from time to time made use of in the general course of the investigations. In such experiments the subaerial factors may, or may not, have been under control. For convenience in treatment they will be grouped as cultural, for the most part carried on outdoors and in laboratory experiments where, as opposed to the first named, the conditions were at least to a certain extent under control.

In experiments with plants outdoors they either were grown in unaccustomed habitats where not only the soil but many other environmental factors were strange, or in the same general habitat with modification of the soil, or they were put in receptacles of various kinds which were filled with the desired experimental soil. In any case,

not only were the conditions of aeration profoundly changed, but other features as well, notably the temperature. Although certain interesting results followed such experimentation, analysis of the results could only be achieved by more intensive and more exact experimental studies in the laboratory.

In the outdoor cultures, alternating vertical columns of fine soil, clay or adobe, and sand were used either in the garden soil or in culture cages. In addition, terracotta and galvanized pipes were also used. In the latter case the soil was moderately fine. There were also cultures in which fine soil only was used, which, by differential irrigation, was kept in part saturated and in part air-dry.

The clay-sand cultures and the culture last referred to were assumed to be unequally aerated, the fine soil or the saturated soil being less well aerated than the dry soil or the soil of a more coarse character. Be this as it may, it suffices to say that plants which developed in the two greatly dissimilar types of soil, as well as those in the soil carrying a large amount of water as opposed to the air-dry soil, possessed roots greatly unlike in the direction and in the extent of growth. But the point to be made in this place is that the detection of this was possible because in certain cultures the columns of unlike soil were vertically placed. In accomplishing the latter, whether in the garden soils or in especially constructed culture boxes, vertical partitions of thin material were used to separate the plot or culture box into the requisite number of divisions which were filled with the respective soils, after which the partitions were removed, leaving the different kinds of soil vertically in direct contact. Plants were grown either wholly on each type of soil or on the line separating them. In the former case a root development characteristic of each soil took place, and in the latter the portion of the root system in the sand developed in a different manner from the portion in the abode.[1]

The culture-box was made of galvanized sheet-iron with three divisions, namely, an upright square compartment at either end with square base, and a long compartment connecting the two, the back of which is vertical and corresponds with the back of the end compartments and the front of which slopes backward from the top, where it has the same width as the compartments at the ends, to a fairly narrow base which thus is somewhat recessed. The top of the three divisions and the bottom are on the same level. The end compartments therefore serve to maintain the central portion in an upright position. The front of the middle part is of glass. The middle compartment has the following dimensions: length 90 cm., width on top 20 cm., width on the bottom 8 cm., and height 60 cm. The front of the bottom of the glass front of the box, therefore, is 12 cm.

[1] W. A. Cannon, Modification of root habits by experimental means. Year Book Carnegie Inst. Wash., No. 17, p. 83, 1918.

farther from the front of the box than the top. This gives a sharp slope, which is sufficient to bring many of the growing roots into contact with the glass where their growth can be observed.

The root-culture box has been used in various ways, including that mentioned above. In this instance bulbs of onion were planted in May and the ground thoroughly wetted. It then was covered tightly with waterproof paper and was not again irrigated during the season. In the last of September the onion plants, which had been growing in sand, or fine clay, or in both sand and fine clay, after the manner of the outdoor experiment referred to above, were removed from the soil. It will be sufficient to remark in this place that the roots in sand and in adobe showed characteristic differences in development, although the method was not competent to reveal the possible cause, which will be commented on in another place.

A digression should be made to remark that the culture-box described, when properly insulated, is of use in investigations on the effect of temperature on root and shoot development. In one such experiment the box was left outdoors in the experimental garden of the Coastal Laboratory in October. Young plants of *Opuntia versicolor*, of southern Arizona, which does not normally produce new shoots even in the warm season at Carmel, were induced to do so under the above conditions, when electric lamps of small capacity were left burning for 6 weeks, one in either end compartment. No growth occurred in the plants midway between the heated chambers, but copious shoot-growth, shoots several centimeters long and with leaves, were formed in the plants near the end of the soil-box. Active root-growth had also taken place. The temperature of the garden air at the time was fairly low, being, as a matter of fact, considerably below that of summer.

In other cultures, with or without homogenous soil, auto-irrigators were planted in opposite ends of the culture chamber for the purpose of providing a continuous gradation in the water-content. Immediately about the irrigators the soil was kept saturated, under which condition gaseous exchange is poor, while at a distance the amount of water is less and the aeration of the soil correspondingly better. Possible differences in soil-temperature are not taken into account. Species of different habit of growth were grown at different distances from the water poles. Positive results were sometimes obtained, either in the way of growth negative to the poles or in indifference to them. In *Brodiæa*, however, unusual succulent development of the main root occurred both in well-wet sand and wet adobe, suggesting that at least in this instance differences in soil aeration may not be the inciting cause. The method is not suitable for exact work, as was also found to be the case in the other analogous cultural methods employed, but it is of interest in bringing out certain differences in

growth as between different species, and, as just shown, in revealing possibilities in growth and development not commonly known or suspected.

The suggestion appears appropriate that a modification of the alternating sand and fine-soil culture method can be devised in which a differential temperature of the soil can be maintained, as was done in certain experiments mentioned above, and a differential water-content also be kept fairly constant, as in the experiments last referred to. Under such conditions interesting differences in reaction of the roots, as shown in many directions, can be expected, and these can be associated in somewhat exact manner with as many factors as may be possible to measure, leaving for definitive results the employment of other methods.

In the second type of outdoor experiment carried out at Tucson, terra-cotta and galvanized-iron pipes, 7.5 cm. inside diameter, and 2.5 meters in length, were filled with coarse soil-sand mixed with a small proportion of adobe—and were maintained in an upright position. The joints of the tubes were fairly tight. The tubes were left standing in metal cans of about 20 liters capacity. Irrigation was effected both by filling the cans with water and by pouring water in the top. Owing to the almost total lack of water-loss from the surface of the soil and the relatively small consumption by the plants grown in them, frequent irrigation was not necessary.

The tubes were planted with cuttings of *Opuntia*, native of the Tucson region, and which in their proper habitats form only shallow root systems. The experiment ran for about 3 years. As to the results, it is sufficient to say in this place that in the case of *O. versicolor* it was found that roots extended to the bottom of the tube and spread somewhat on the bottom of the metal can. The root system thus was over 2.5 meters in length. The formation of few and unimportant laterals in the middle part of the tubes, that is, away from either end, and the fairly rich branching near and at the bottom, lead to the supposition that the one was in part the result of an insufficient oxygen-supply and the other was made possible by good aeration. Unquestioned conclusions, however, could not be drawn, for the reason that the actual partial pressure of oxygen in the tubes was not known, neither was the amount of carbon dioxid determined, and, moreover, it was determined in other experiments that the roots of these species of *Opuntia* require fairly high soil temperatures for growth. Such temperatures would be experienced by the cultures during a portion of the year for the reason that they were freely exposed to the sun part of each day.

Other methods directly associated with the growth of roots in connection with the substratum, not described elsewhere, may be briefly characterized. Of these one concerned itself with the growth of

Krameria seedlings in potato tubers and in flattened joints of *Opuntia discata*, with results to be mentioned elsewhere, and another had to do with the effect on germination and early growth of germinating seeds which had been covered by different depths of soil. Soil depth was found to directly affect the relative length of root and of hypocotyl. This is also referred to in another place.

In other experiments a root clinostat was devised by which to observe the course of roots in soil when the effect of gravity was neutralized. Circumstances, however, have prevented the carrying on of this line of work. However, as devised, the clinostat had the following important features: Galvanized-iron receptacles, holding about 20 liters, were removably attached to metal bases, which were so connected with an actuating motor that they were caused to revolve slowly in any position between the horizontal and the vertical. Although certain results were obtained from this apparatus as constructed, it was learned that it was desirable to utilize somewhat smaller soil containers. Accordingly, without otherwise modifying the apparatus, the containers were made as four similar quarters, two of which could be used at one time by so placing them that the balance was not seriously disturbed, while at the same time two of the quarter tins were left empty. In practice, however, it was found better to use a single but smaller container. In any event, a fairly large motor is essential, owing to the difficulty in starting the load in case the motor, for any reason, stops.

Although the apparatus and methods above described were designed especially for observing the reaction of roots, as shown by a variation in the rate of growth, to differences in the aeration conditions, they also are in part useful in studies on the relation of the root to the shoot, particularly as regards the influence on the rate of growth of the shoot of an excess of carbon dioxid, or a diminished supply of oxygen in the atmosphere of the soil. Opportunity had not yet been afforded to make great use of the apparatus referred to, but an account of it may nevertheless be appropriate in this place.

In observing the growth both of the root and of the shoot at the same time, when the general method of root control in use is employed it is necessary to have a means of determining shoot-growth which is at once accurate and convenient to handle. Such a means has been found in the especially devised auxanometer and auxograph, illustrated in figures 3 and 4. In both types of measuring apparatus the attachment is directly to the culture tube, so that when and if desirable, as, for example, to observe the growth of the root, the entire apparatus, including the culture tube, can be easily removed from the thermostat without in any way disturbing the continuity of the experiment. In addition to this, the culture can be treated in every way as if the shoot were not under observation.

The auxanometer (fig. 3) is of simple construction, which is well illustrated by the figure. The degree of magnification of the shoot-growth can be easily changed by modifying the relative lengths of the index arm and of the shorter arm to which the shoot is attached. In the figure each space on the dial is approximately equal to 1 mm. growth.

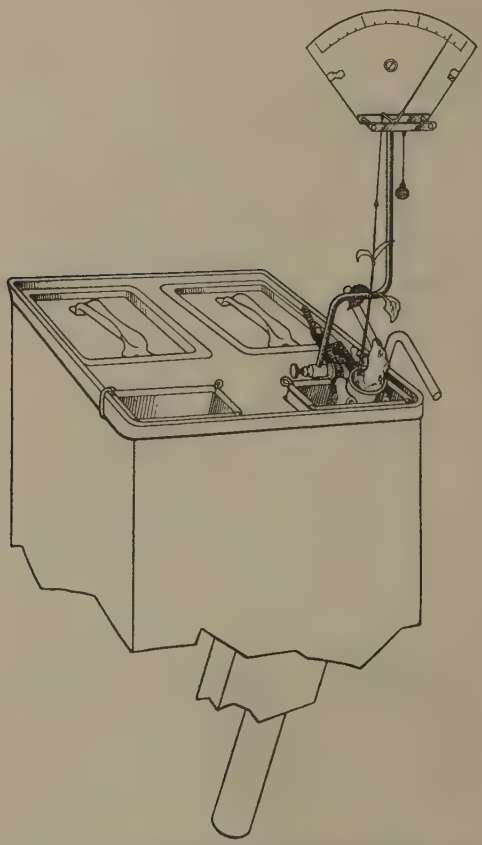

FIG. 3.—Auxanometer used in studying shoot-root relations, shown attached to culture tube, and culture tubes, together with auxanometer, can be removed from thermostat for observation without disturbance to the culture.

Although the auxograph (fig. 4) is somewhat more complicated than the auxanometer, the leading points in its construction can be easily understood with the aid of the figure. It consists essentially of a drum which is removably attached to the hour spindle of a small-sized alarm-clock of well-known make, and which in turn is securely

attached to a metal base. True rotation of the drum is attained with the use of a removable pin which projects downward through a metal frame. The drum is an aluminum box with tightly fitting cover which is fitted to the clock spindle. By raising the centering pin above

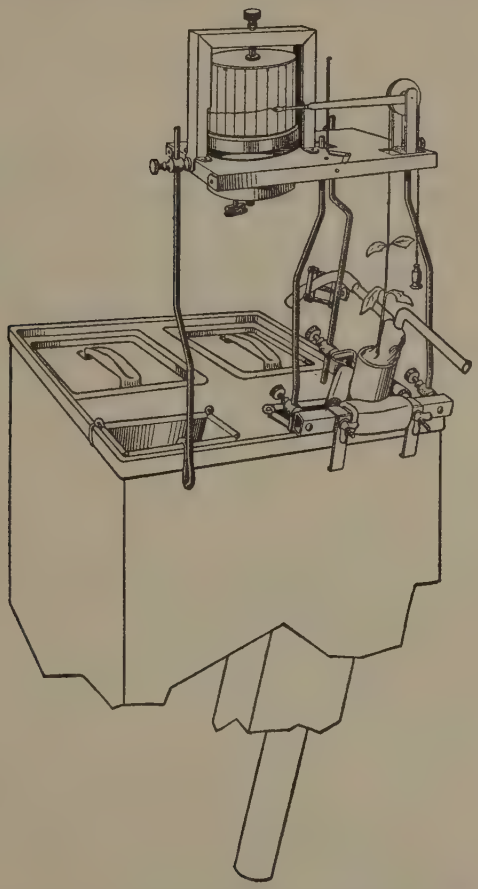

FIG. 4.—Auxograph used for the same purpose as auxanometer. Instrument is attached to culture tube, as indicated in figure, but rests on the thermostat without being attached to it. Like the auxanometer, therefore, it can be removed with culture tube from the thermostat without disturbing the culture.

referred to, the drum can be easily removed for the attachment of a paper record. To accomplish the latter, a vertical slit, not shown in the figure, is made in the drum, in which the two ends of the record-slip are placed with the ends inside. The paper is kept tight either by sharply folding backward the ends of the record or by placing against

them a spring clamp. As for the balance of the apparatus, it need only be said that the recording arm is permanently attached to a pulley with a roughened groove in which lies a light chain, as indicated in the figure. The pulley operates in a U-shaped swivel attachment which is rotated somewhat when the recording arm is raised off the record. The chain, which rides in the pulley, is provided with a counterweight. It will be seen that by the proper manipulation of the pulley and the chain any desired position of the recording arm and of the recording pen can be had.

In both auxanometer and auxograph attachment to the shoot is by means of a silk thread or fine fishing-line, which may be shellacked to prevent the absorption of moisture and possible consequent change in length.

An account of two or three experiments, which are not given as being conclusive, will be sufficient to illustrate the working of the apparatus and method. On September 20 an auxograph was attached to the shoot of a variety of sweet corn which was growing in a culture tube and in a thermostat running at about 25° C. The record began tracing at $10^h\ 30^m$ in the morning. The root was in normal soil atmosphere. At 3^h in the afternoon the culture was given cylinder nitrogen containing about 0.5 per cent oxygen. A normal rate of growth of the root was recorded to have taken place up to this time and slight growth of the shoot. The nitrogen was given continuously until the following afternoon. At 10^h, when the experiment was ended, it was seen that no growth of the root had taken place, but that the shoot had increased about 7 mm. in length, most of which had occurred during the hours of darkness.

On September 21 the root of seedling barley was given cylinder carbon dioxid and the administration was continued 40 hours. No root-growth occurred. Of this time, however, shoot-growth went on at a rather slow rate for about 30 hours, fairly rapidly during the first half of the time and more slowly afterward, and then growth ceased.

In the preceding paragraph the magnification of growth was about 5, which was not sufficient to permit the detection of small growth changes. In another experiment a magnification of about 20 was used when it was desired to closely observe the direct effect on growth of an excess of carbon dioxid in the soil. In this case shoot-growth was demonstrated while the root was in "normal" soil atmosphere at the beginning of the experiment. The experimental atmosphere was then given. As a result, it was observed that within an hour following this the rate of growth of the shoot began to fall off and shoot-growth wholly ceased within 6 hours.

In table 3 is given root and shoot growth of *Opuntia versicolor* with "normal" soil aeration. The shoot-growth is computed from the auxographic record.

TABLE 3.—*Shoot and root growth in Opuntia versicolor.*

Time.	Shoot-growth.	Root-growth.	Temp.
	mm.	*mm.*	° *C.*
$7^h\ 30^m$ a. m. to $5^h\ 30^m$ p. m.....	1.2	1.5	21 to 26.5
6 30 2 30	1.0	.6	13.5 29
8 12 2 00	1.6	2.0	20 30.5
7 45 3 45	2.1	.7	20 31
7 45 3 45	2.0	2.0	20 31
7 45 3 45	1.3	2.9	20 31

In the last experiment, during the same daylight hours, the growth of the shoot thus was slightly greater than that of the root, although the daily growth of root and shoot did not usually run parallel. No observations were made on root-growth during the hours of darkness. Further exposition of the method is not necessary in this place.

DIFFERENCES BETWEEN NITROGEN AND HELIUM AS INERT GASES IN ANAEROBIC EXPERIMENTS ON PLANTS.[1]

INTRODUCTION.

In the large amount of reported experimentation on the behavior of plants and animals in the absence or deficiency of oxygen, one of three procedures has usually been employed to obtain an oxygen-free space. The first, historically, is the use of a vacuum. This has well-known disadvantages in the disturbance of gas-pressures within the living tissues and has largely been superseded by one of the other two procedures, namely, the use of either nitrogen or hydrogen as an "inert" gas with which the normal atmospheric air is replaced. The other gases occasionally used, such as nitrous oxid and carbon dioxid, are known or suspected to have specific effects on protoplasm and have become little used in recent work. Hydrogen also is under suspicion, because of its active power of chemical reduction and because of its known participation in the metabolism of certain bacteria. Accordingly, recent work on anaerobiosis has made use, nearly exclusively, of nitrogen as the medium, supposedly inert, with which the oxygen and other active gases are diluted or replaced.

In the experiments on soil aeration with which the writers have been engaged, nitrogen has been used as the inert gas. Experiments previously reported[2] have been directed to the discovery of the reactions of plant roots in soil the normal interstitial atmosphere of which has been replaced with nitrogen or with known mixtures of nitrogen and oxygen. This experimentation need not be reviewed further than to say that species vary widely in their responses to deficient soil oxygen, but that several of the species which have been tested show clear and immediate injuries, resulting finally in the death of the roots and of the plant. Although these results were ascribed confidently to the effect of mere oxygen deficiency, there remained, theoretically, a chance of error in the possibility that nitrogen was really not entirely inert toward the organic complex of the soil. Like hydrogen, nitrogen is known to take part in the metabolism of certain lower organisms, being absorbed by some and set free by others. Even if there were no effect of elemental nitrogen directly on the plant roots, there was possibility that an effect of it on the content or products of the microflora of the soil might react indirectly on the higher plant. Accordingly it became desirable to check the results obtained

[1] This chapter was prepared by E. E. Free.

[2] Cannon, Amer. Jour. Bot. 2: 211–224 (1915); Livingston and Free, Johns Hopkins Univ. Circular, March 1917, pp. 182–185; Cannon and Free, Science (n. s.) 45, 178–180 (1917).

with nitrogen by the use of some other gas which could be assumed to be inert.

With the development, as a matter of war preparation, of means of procuring helium on a large scale, this element became available and, through the courtesy and assistance of the Bureau of Steam Engineering of the U. S. Navy, a cylinder of this gas was supplied to the Coastal Laboratory, Carmel, California. We wish to express our thanks and appreciation for this co-operation on the part of the naval authorities, without which this investigation would have been impossible.

The first experiments with helium showed that this gas, drawn directly from the cylinder, gave effects distinctly different from the effects secured in parallel experiments with nitrogen. The nitrogen used was the ordinary commercial product supplied by the Linde Air Products Company, and was of the same character and source as had been used by us in experiments covering more than 5 years. It had been tested frequently for impurities and compared with nitrogen made directly from the atmosphere. However, these tests and comparisons were repeated, with the results that no toxic impurity was found and that nitrogen directly from the air showed the same differences from helium as were shown by the cylinder nitrogen. The helium was then subjected to similar tests and no toxic or stimulating impurity was found. It was known that both gases, as drawn from the cylinders, contained small percentages of oxygen, the nitrogen approximately 0.5 per cent and the helium approximately 0.2 per cent. These traces of oxygen were not at first considered important. It will be noted that the percentage of oxygen in the more toxic gas, nitrogen, was over twice as great as in the less toxic one. However, it was found, ultimately, that these small percentages of oxygen lay at the root of the apparent difference between the gases. When the traces of oxygen were removed as completely as possible by alkaline pyrogallol, the roots behaved similarly in the helium and in the nitrogen. So far as was determinable, the two gases, each free of oxygen, were equally "toxic."

The details of the experimentation thus outlined are described in this section and lead to the conclusion that the apparent difference between helium and nitrogen lies in the greater rapidity with which oxygen diffuses through helium. When the percentage of oxygen is small this rate of diffusion appears to be a large, and frequently the largest, factor in controlling vital activity. So far as we are aware, this is a matter not taken into account by any previous investigator of partial or complete anaerobiosis.

EXPERIMENTS WITH POTATO.

One of the methods previously employed by us in the study of the effect of soil aeration on plant roots may be described as follows:

The plant is grown in soil in an ordinary tin can, such as is used in preserving fruits (fig. 5). These cans are obtainable commercially in various sizes, of which we have used commonly either the 2-quart size, which is approximately 13 cm. in diameter by 13 cm. high, or the 1-gallon size, which is approximately 15 cm. in diameter by 18 cm. high. The plant being well established, a tin top, with a central hole for the plant stem, is soldered tightly to the tin, and a seal is built up around the plant stem, using ordinary sealing-wax for an external

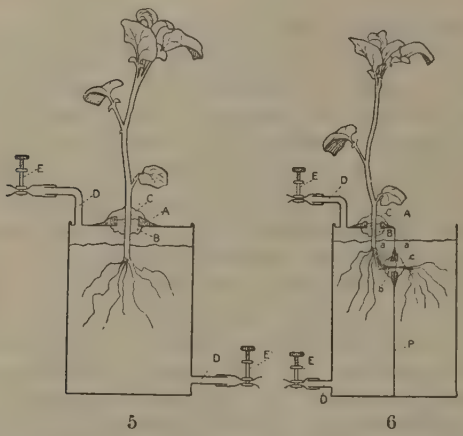

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FIG. 5.—One form of apparatus used in studies on potato and other plants. The plant is sealed in the metal container with sealing-wax or de Khotinsky cement (A), and with grafting-wax (C). Cotton (B) may be left about the stem. More complete account of this apparatus, as well as of apparatus shown in later figures, is given in the text.

FIG. 6.—Metal container, similar to that shown in figure 5, arranged for differential studies on roots. Inlet and outlet tubes (D), of lead, are soldered to container. In the arrangement the crown of root and a portion of root system are included within sealed portion. As in the case of the container in figure 5, various sealing material may be used, including, in this instance, plasticine.

collar sealed to the tin top, and melted grafting-wax inside of this and against the plant stem. The foliage and aerial parts of the plant project into the general atmosphere, while the roots and soil are inclosed in the sealed tin. Lead tubes soldered to the tin provide for inlet and outlet of gas and may be closed by rubber tubes and pinchcocks or by plugs of solder. Manometers, thermometers, tubes for watering, and the like may be inserted similarly, either through soldered lead connections or through glass tubes sealed to the tin by sealing-wax or de Khotinsky cement. This technique has been previously applied by us to the ordinary potato, *Solanum tuberosum* var. "Salinas-Burbank," and it had been found that the replacement of the soil air by cylinder nitrogen, containing 0.5 per cent oxygen, caused

immediate and serious injury, characterized by the death and decay of the roots and subterranean stems, and resulting in wilting and ultimate death of the plant. This is a behavior quite common among the plant species which are injured by deficiency of soil oxygen.

Comparison of helium and nitrogen under this technique was first made by two preliminary experiments run under conditions of the greenhouse at Carmel, which is kept largely open during the summer and differs little, except in light, from the conditions out-of-doors. During the experiment the temperatures in a similar culture tin on the same bench varied from 11° C. to 24° C. In one of the experiments the potato plants used had been grown from tubers of the previous year in the usual fashion and had the old tuber still attached to the root system. These plants were 20 days old and were each 15 cm. high. The two plants used were selected as being as nearly similar as could be determined by inspection. The other experiment made use of two plants which had been started from cuttings, without any older tuber. Accordingly, these had no tubers except such as they themselves had formed. These plants were 21 days old, dated from the transplanting of the rooted cutting and were also 15 cm. high and selected for apparent similarity. One plant of each set was treated with cylinder nitrogen, 0.5 per cent oxygen, and the other with cylinder helium, 0.2 per cent oxygen. The results are given in table 4.

TABLE 4.—*Wilting and growth of potato plants with roots in nitrogen and helium.*

Time after beginning of experiment (days).	Plants with old tuber.		Plants without old tuber.	
	Roots in nitrogen.	Roots in helium.	Roots in nitrogen.	Roots in helium.
4	Unchanged	Unchanged	Unchanged	Unchanged
5	Do.	Do.	Slightly wilted	Do.
6	Slightly wilted	Do.	Distinctly wilted	Slightly wilted
8	Distinctly wilted	Slightly wilted	Badly wilted	Badly wilted
9	Badly wilted	Badly wilted	Do.	Do.
10 (final)	Roots and tuber entirely dead and decayed.	Roots and tuber entirely dead and decayed.	Roots entirely dead and decayed.	Roots entirely dead and decayed.
Number of days required for perceptible wilting.	6	8	5	6
Growth during experiment: Elongation of stem (mm.)	0	10	0	15
Per cent	0	7.4	0	11.5
Average elongation of 2 youngest leaves (mm.)	0	0	4	10.5
Per cent	0	0	11.4	58.3

It is apparent from table 4 that in both cases perceptible wilting occurred earlier in the plant treated with nitrogen than in the one

treated with helium. "Unchanged" in the table means merely that no change could be detected by visual observation of the shoot. What may have happened to the root is unknown. When the cultures were finally taken down, after 10 days, the roots of all plants were found to be dead and decayed exactly as had been observed previously with plants in nitrogen. There was no perceptible difference in root condition between the cultures in helium and those in nitrogen. Throughout the experiment, after wilting became perceptible at all, the plant in nitrogen was always distinctly more wilted than that in helium. In this case, as in all others where a factor of personal judgment was involved and was regarded as of any importance, the judgment of the experimenter was confirmed or corrected by the judgment of at least one other person not acquainted with the conditions of the experiment or informed concerning the judgment or expectation of the experimenter.

There are also included in the table figures concerning the growth made by the plants during the experiments. While growth is too complex a matter to be relied upon as an index of injury, it will be noted that the differences in growth are parallel, so far as they go, with the differences in time of wilting.

The firm establishment of the observed difference between the cylinder helium and the cylinder nitrogen being regarded as of considerable importance, the experiments with potato were twice repeated, once in a glass-walled constant-temperature cabinet kept at 24° to 25° C., and again in the underground constant-temperature room of the Coastal Laboratory, which is constant during the summer at 16° C., with a daily variation of less than 0.5° either way. This latter experiment was necessarily run in the dark. In each set there was included a culture in a mixture of 50 per cent nitrogen and 50 per cent helium, by volume. All plants were grown from old tubers in the normal manner, were 25 to 30 days old and approximately 15 cm. high, and were selected for similarity, as in the former cases. The results are given in table 5.

It will be seen that the results are entirely consistent with those given in table 4. The plants in nitrogen show injury distinctly sooner than those in helium, and those in the 50–50 mixture of the two gases are, on the whole, intermediate between the other two. The greater rapidity of injury in the cultures at 24° to 25° C., is as would be expected, but too much can not be made of such differences between non-parallel experiments. Our previous work on injury by deficient soil aeration has furnished many examples of the complex manner in which such injury, and especially its indication by wilting, depends upon the entire complex of internal and external factors, notably transpiration, food-supply, and size of root system.

The condition of the root systems at the end of the experiment at

16° C. is believed to be especially significant. This experiment was stopped, purposely, before the wilting of the shoots was complete. As noted, the decay of the roots was found to be incomplete also, and the differences observed in the completeness of this decay are in line with the other differences observed. This difference in the root systems was unmistakable, the coherent and apparently healthy portion of the root system from helium being probably ten times as large as that of the plant from nitrogen.

TABLE 5.—*Wilting and growth of potato plants with roots in nitrogen and helium, at 24° to 25° C. and at 16° C.*

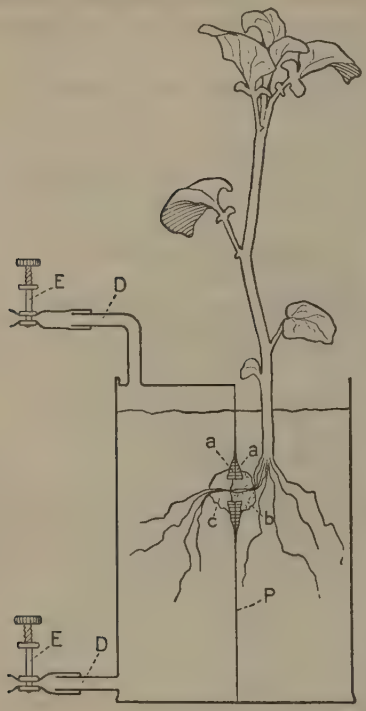
Time after beginning of experiment (days).	Roots in nitrogen.	Roots in 50 p. ct. nitrogen, plus 50 p. c. helium.	Roots in helium.
Cultures at 24°–25° C.			
1	Distinctly wilted.	Possibly slightly wilted, but uncertain.	Unchanged.
2	Badly wilted.	Distinctly wilted	Slightly wilted.
3[1]	Badly wilted, roots dead and decayed	Badly wilted, roots dead and decayed.	Distinctly wilted. Roots dead and decayed.
Cultures at 16° C.			
2	Unchanged.	Unchanged	Unchanged.
3	Very slightly wilted.	Do.	Do.
4	Distinctly wilted.	Possibly slightly wilted, but uncertain.	Do.
6	Distinctly wilted, root system largely dead and decayed.	Slightly wilted, root system partly dead, but less completely than plant in nitrogen.	Slightly wilted, root system partly dead, about as completely as plant in nitrogen and helium.
Growth:			
Stem, mm.	10	20	25
Per cent	8.3	12.9	16.7
2 youngest leaves, mm.	3	4	4
Per cent	10.5	14.3	14.5

[1] No measurable growth during the experiment.

After the completion of the experiments reported in table 4 and table 5, the shoots of the injured plants were cut off above the portion of the stem into which decay might have spread from the roots, the dead or dying leaves were removed, and the shoots were put in Knop's solution. All of them revived and made new roots from the cut end of the stem, thus indicating that the injury to the plant had left no permanent toxemia or other effect in the shoot. It should be said, in passing, that the rooting of potato cuttings in nutrient solution in this fashion is easy under the equable climatic conditions of Carmel and was commonly employed by us to obtain plants not attached to tubers.

In the experiments of table 5, the production of carbon dioxid in the soil was followed by analysis of gases drawn from the tins, and in the experiments of table 4 this was followed both by analysis and

by the reading of mercury manometers inserted in the tins. Although these records served as precaution against undetected leakage, the results as to carbon dioxid produced are not consistent. In the experiments in the open greenhouse more carbon dioxid was produced in nitrogen than in helium. In the experiments at controlled tempera-



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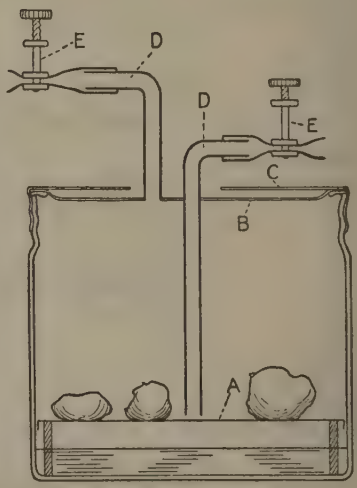

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FIG. 7.—Apparatus similar to that shown in preceding figure, but arranged so that root crown and a portion of roots are exposed to normal soil atmosphere, while the remainder is under the seal and subject to the experimental soil atmospheres used.

FIG. 8.—Arrangement of apparatus for studying aeration relations of potato and other plants, in which a glass jar with metal screw-top of two parts, *C* and *B* is used. Inlet and outlet gas tubes of lead are soldered to part *B* as indicated and project through *C* as shown. A rubber sealing-ring is fitted between metal top and glass portion of container.

tures this condition was reversed and the production of carbon dioxid was greater in the helium. This discrepancy must be elucidated by further work before discussion of this feature of the matter will be justified.[1] Since the production of carbon dioxid in the soil is known

[1] Some of the earlier data on carbon-dioxid production have been published already. Cannon and Free, Carnegie Inst. Wash. Year Book for 1919, p. 85.

to depend not only upon the aerobic or anaerobic respiration of the plant roots but upon the numerous and varied activities of micro-organisms, the fact that the results are not concordant does not impress us as constituting any qualification to the consistency of the results as to the injuries to the potato roots themselves.

In addition to the experiments with potatoes in soil, an experiment was run on potatoes growing in Knop's solution. The technique was substantially similar to that already described, but glass jars, such as are used in preserving, and of 1 quart capacity, were used instead of the tins. These jars are procurable commercially, provided with metal

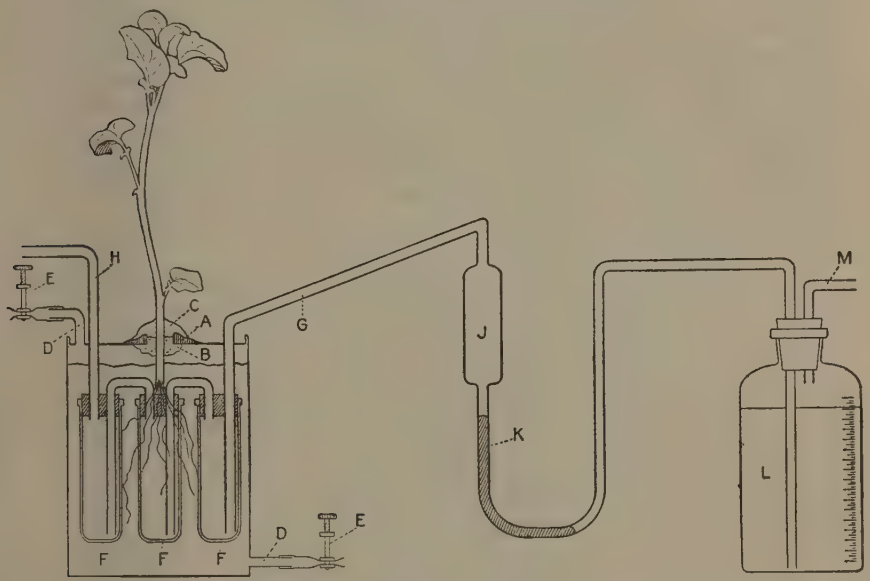

FIG. 9.—Apparatus in which auto-irrigating apparatus is included in culture chamber. Metal container like that shown in figure 5 is used. This is fitted with inlet and outlet gas tubes, *D*, and porous clay tubes, *F*, connected in series with water chain, *H–M*, with mercury valve. Water reservoir is shown at *L*.

tops adapted to screw on to the upper edge of the jar, the top being sealed to the jar edge by a rubber washer. This procedure was employed exactly as in household preserving, a hole being punched in the metal top for egress of the plant stem and the seal around the stem being made with sealing-wax and grafting-wax as before. Lead tubes soldered into the metal top provided inlet and outlet for gases and a heavy paper shield around the jar protected the roots from light. The plants used were potato cuttings previously rooted in Knop's solution and which had never been in soil or attached to tubers. The shoots were approximately 7 cm. long and had been in Knop's

solution for 24 days. The root systems were ample in size and apparently normal for water-culture plants. The two plants were selected for similarity. The Knop's solution had the usual composition of 4 parts calcium nitrate, 1 part magnesium sulphate, and 1 part sodium chloride, and was used in a total concentration of 4 grams per liter. This solution had been found exceptionally favorable for the rooting and growth of potato cuttings. A portion of this solution was boiled for 5 minutes, the water-loss was made up by boiled distilled water, and the solution was poured quickly into a clean culture-jar. The metal top was screwed down quickly. The seal was completed about the plant and the air above the culture solution in the jar was swept out by a rapid stream of nitrogen or helium. A rubber gas-bag containing the desired gas was then attached to one outlet of the jar, the other outlet was closed, and the culture solution left under the slight positive pressure of the gas produced by the elastic tension of the bag. With this technique parallel cultures under nitrogen and helium gave the results presented in table 6. As before, the gases were as drawn from the cylinders and contained, therefore, the usual small percentage of oxygen.

TABLE 6.—*Injury to potato roots in non-aerated Knop's solution, under nitrogen and helium.*

Time after beginning of experiment (days).	Roots in solution under nitrogen.	Roots in solution under helium.
4	Unchanged	Unchanged.
5	Slightly wilted and some leaves dead at tips; roots drooping and apparently lacking in turgor.	Same as plant under nitrogen.
7	Appearances the same, but wilting and injury more advanced.	Do.
10 (final)	Same, but injury more advanced; root system found to be entirely dead and largely decayed.	Do.

Previous experiments with this same technique and with nitrogen and other gases had shown injuries under nitrogen similar to those described in table 6. It had been found, also, that plants grew normally in cultures of this sort when the gas above the solution was air or oxygen; that carbon dioxid, alone or mixed with 20 per cent of oxygen, was injurious; and that the results with cylinder nitrogen were in all ways the same as results with nitrogen procured directly from the air. It had been found also that the shoots of injured plants were able to recover and set roots when cut off and put in Knop's solution, and that the non-aerated solution in which injury had occurred had acquired no toxic properties thereby, but was entirely suitable, when again aerated, for the growth of new, uninjured plants.

In the light of these facts with reference to the technique in general, it is apparent from the data of table 6 that the difference in the rapidity of anaerobic injury to potato roots in atmospheres of nitrogen and of helium, which difference is observed in soil cultures, is not perceptible when the cultures are in nutrient solution. We shall discuss later the probable explanation of this fact.

EXPERIMENT WITH COLEUS BLUMEI.

In experiments previously reported,[1] the reactions of *Coleus blumei* to deficient soil aeration were investigated in some detail, using nitrogen as the inert gas. It was found that this plant is exceptionally sensitive to deficiency of soil oxygen. For comparison with these earlier results a test was made of the comparative behavior of this species in nitrogen and in helium. The technique was the same in all details as that described for soil cultures of potato. The plants were of commercial nursery stock, transplanted into tins 18 days before being used, and were well established in the tins. The two plants used were each approximately 20 cm. high, were of the same strain of leaf color, and were selected for similarity. The cultures were kept in the constant-temperature cabinet at 24° to 25° C. After two days both plants were slightly wilted. The wilting gradually increased until the eighth day, when the cultures were opened and the roots examined. The root systems of both plants were dead and largely decayed. The stems were cut off above the decay and put in Knop's solution, where in 10 days they had recovered their turgor, and in 17 days had started new roots. At no time during the experiment was there any perceptible difference between the culture in nitrogen and that in helium. The development of carbon dioxid in the soil was followed both by manometer readings and by analysis, and was substantially the same in both cultures. It is evident that the differences between nitrogen and helium which are exhibited with potato do not occur with *Coleus*.

BLANK EXPERIMENTS.

The experiments with potatoes in sealed tins having led to such clear differences between cultures in the two gases, it became desirable to make sure that these differences were not due to errors of experimental method. Chemical and biological tests, described later in this paper, rendered the presence of any toxic or stimulating impurity in the gases very improbable. It was felt that the observed differences with potato were of such magnitude, and so consistently in the same direction, that they could not be ascribed to accidental differences in the reactions or initial conditions of individual cultures. Further-

[1] Livingston and Free. Johns Hopkins Univ. Circular, March 1917, pp. 182–185.

more, our previous work on soil aeration had led us to expect that such accidental variations, while wide and frequent between cultures run at different times or under different environmental conditions, were not to be expected in strictly parallel cultures of material selected as similar. In all previous experiments, parallel conditions and synchronism of cultures has meant corresponding results.

The only other experimental error foreseen as probable was the possibility of a greater inward leakage of atmospheric oxygen into helium than into nitrogen. A priori, there is no reason to expect this. In all of our cultures, attachment of a bag of gas under pressure or the internal evolution of carbon dioxid created a slight excess of pressure inside the tins. Accordingly, any actual molar leakage of gas through holes in seals or tins would be outward, not inward. Such leakage would be immediately detected by change in the manometer reading, and in one case such a leak did occur in the grafting-wax seal and was detected and repaired before the internal pressure had fallen to that of the atmosphere. There remained the possibility of leakage through the rubber tubes used for connections, or through the plastic materials of the seal about the plant. The knowledge of the passage of gases through rubber or other colloidal plastics rests mainly upon the early investigations of Graham.[1] The rate of passage is not controlled, as in gas diffusion, by the molecular kinetics of the gas, but depends upon a mutual relation, analogous to solubility, between the particular gas and the particular plastic in question. Thus carbon dioxid passes through rubber nearly three times as fast as does hydrogen, though the diffusion of hydrogen in space or into other gases is three to five times faster. In the particular case here concerned the significant matter is the inward passage of oxygen. Accordingly, since the solubility of this oxygen in the rubber or in the seal will be essentially the same in all cultures, and since the external concentration of oxygen in the atmosphere is constant, there is no reason to expect any greater passage of oxygen into the helium cultures than into the nitrogen ones.

Although this reasoning seemed to be entirely cogent, it was thought best to test the matter directly. Accordingly, two similar gas bags of elastic rubber were filled, one with cylinder nitrogen, the other with cylinder helium, and were exposed to the atmosphere in the laboratory. In each case the outlet tubes were closed with screw pinchcocks of usual pattern. As was expected, there was a certain passage of oxygen into each bag and also an escape of helium and of nitrogen outward. Because of this outward passage of gas, both bags tended to collapse, and it was found that the helium bag collapsed faster than the nitrogen one. Evidently the helium escaped

[1] J. H. Poynting and J. J. Thompson. Textbook of Physics: The properties of matter, 6th edition, p. 203, 1913.

through the rubber faster than did the nitrogen, but this does not necessarily mean a greater "solubility" of helium in the rubber. The helium was escaping into a space, the atmosphere, in which the concentration of helium was negligible, while the nitrogen was escaping against a surrounding nitrogen pressure 80 per cent of the atmospheric. For present purposes this more rapid collapse means only that the analytical data for oxygen inside the bags must be corrected accordingly. Since the oxygen in the bags is measured as a volume percentage of the total gas in them, the oxygen percentage will suffer an apparent but illusory increase due merely to the decreased amount of other gas in the bag. The data obtained directly in the experiment, with the same values corrected for the progressive collapse of the bags, are given in table 7. The corrections are made by means of measurements of the volumes and pressures of the bags made when the samples were taken for analysis. It is apparent that within the limits of accuracy of the experiment the passage of atmospheric oxygen through rubber into helium is not importantly more rapid than its similar passage into nitrogen.

TABLE 7.—*Passage of atmospheric oxygen into rubber bags filled with nitrogen and with helium.*

Time of exposure to atmosphere.	Oxygen in bag as obtained by anaylsis.		Oxygen in bag, corrected for collapse of bag.	
	In nitrogen.	In helium.	In nitrogen.	In helium.
hours.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
0	0.4	0.2	0.4	0.2
25	.6	.5	.6	.4
43	1.1	1.1	1.0	.9
144	3.6	4.1	3.5	3.4
260	5.6	8.3	5.0	5.4

In order to test the passage of oxygen through the plastic materials, sealing-wax and grafting-wax of the seal about the plant stem, two empty tins were prepared, sealed, and equipped in all details like a plant culture, but with glass tubes 9 mm. in outside diameter taking the place of the plant stem. These tubes were sealed at both ends in the flame and were roughened with a file at the points where the seals were attached to them. One tin was filled with cylinder nitrogen the other with cylinder helium. After 12 days the gas in each tin was analyzed and was found to contain, in each case, 2.3 per cent of oxygen. As before, there was a slightly greater escape of helium than nitrogen, the final reading of the manometer on the helium tin being 1.4 mm. below atmospheric pressure as against 0.4 mm. below atmospheric pressure on the nitrogen tin. In spite of the slightly greater inward pressure to which the helium tin was thus subjected, the quantity of oxygen entering it was not substantially greater than entered the nitrogen tin. Both of these tins were equipped with closed

rubber connections, like the majority of the actual cultures. Accordingly, the inward passage of oxygen was not entirely through the grafting-wax seal. Nor is the actual quantity of oxygen which entered to be regarded as bearing any necessary quantitative relation to the possible leakage of oxygen into other cultures. The experment was designed to test any differential leakage into nitrogen and into helium, and is not regarded as adequate for any other purpose. It is obvious that such a differential leakage is not only improbable in theory, but was not discovered in fact.

EXPERIMENTS WITH CANADA FIELD PEA.

The experiments described in the foregoing sections of this paper established merely that the gases as drawn from the two cylinders did not behave similarly when used as soil atmospheres for the roots of potato. For the further elucidation of the nature of this difference, use was made of another species and another technique. A method of investigation previously employed by one of us[1] in the study of root-growth makes use of seedlings grown in sand in glass tubes 45 cm. long and 2.5 cm. to 3.0 cm. in internal diameter. If these tubes are kept in a position inclined about 30° from the vertical, the geotropic tendency of the roots brings them to the inner surface of the glass, where their growth-rates can be directly observed and measured. For experiments on soil aeration these culture tubes are corked at the lower end. At the upper end a seal is made about the plant stem with grafting-wax or with plasticine. Small glass tubes projecting through this seal provide for inlet and outlet of gases and for watering, when necessary. The tubes are placed, still in inclined position, in thermostats which may be kept at constant temperature. In the experiments here described, the sand in the culture tubes was wetted with Knop's solution of the same composition and concentration specified above.

Seedlings of Canada field pea were grown in these culture tubes from seed selected for similarity of size and condition, and the seedlings were again selected for similarity and for visibility and good condition of roots before being sealed. After sealing, the tubes were placed in thermostats at 23° to 24° C., and the desired gases passed through them. The tubes were protected from light. In some cases the desired gas was merely run through the culture tube until all air was swept out. The outlet was then closed, leaving in the culture tube a static atmosphere of the gas under test. As in the case of the experiments with tins, there was always a slight positive gas-pressure inside the tubes. Any accidental leakage would thus be outward, not inward. In other cases the gas in the tube was changed at more or less frequent

[1] Cannon. Amer. Jour. Bot. 2: 211–224, 1915. See also p. 14 of this work.

intervals, fresh gas of the same composition being swept through for a sufficient time to replace completely the gas previously in the tube. In still other cases a slow stream of gas of the desired composition was passed continuously through the tube, the rate of passage varying in different experiments from approximately 1 liter to approximately 3 liters per hour. In the following paragraphs these three methods of maintaining the desired gas composition within the tubes are designated respectively as "static," "changed," and "streaming." In some experiments rubber gas-bags were employed in handling and storing the gases. In others, metal gasometers were employed. There was no perceptible difference in the results. Care was taken, of course, to use rubber gas-bags only under such conditions that the known passage of small amounts of oxygen through the rubber would be without significant influence on the experiment.

The results obtained by use of the cylinder gases on Canada field pea according to this method are presented in table 8. As before, the gases contain the small percentage of oxygen present in them as supplied. The figures give the growth of the main root or tap-root, in millimeters, during the period between observations. The first left-hand column of the table gives the hours elapsed from the beginning of the experiment; the second column gives the hours covered by the particular period, between observations, during which the growth occurred. Thus in the case of the first plant noted, namely, the plant in a static atmosphere of nitrogen, third column of table 8, the first observation was 17 hours after the start. The period was therefore 17 hours long and the growth was 5.0 mm. The next observation was 24 hours after the start, the period was therefore 7 hours, and the growth was zero. The word "dead" in the table indicates that the tip of the main root had visibly altered in color and turgor. The normal root-tip is slightly brownish. When it dies this color fades to white, and the tip, or the root just back of the tip, shrivels. The appearance is quite characteristic and is unlikely to be mistaken, but in all experiments here reported the observation of death of the root was confirmed by passing oxygen through the culture tube and observing the root for 48 hours or more, to see if growth was renewed. When this is done, lateral roots arising from near the juncture of the stem frequently continue growth and new roots sometimes start, even when the main root is dead. In no case, however, did we observe the revival of a main root which had exhibited the described appearance of death.

The outstanding feature of the data of table 8 is the fact that growth continued in helium and stopped in nitrogen, this being in agreement with the differences already described in the case of potato. At the end of the 48-hour period the tubes with helium (experiments Nos. 4 and 5 of table 8), were swept out with oxygen. The roots continued

normal growth. In the case of the mixtures of nitrogen and helium the results are not entirely consistent. In one case No. 7, growth continued normally and the root also grew normally when oxygen was supplied at the end of the 48-hour period. Two of the roots, (Nos. 6 and 8), died during the experiment. The fourth culture, No. 9, was growing at the end of the 48-hour period and was then put in a static atmosphere of the same gas mixture. The root grew 1.0 mm. in the next 16 hours. In the following 8 hours there was no growth, and after 16 hours more the root was dead.

TABLE 8.—*Growth of roots of Canada field pea in nitrogen and in helium.*

Exp.		Nitrogen.			Helium.		Nitrogen 50 p. c. plus helium 50 p. c.			
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9
Time from beginning.	Time of growth period.	Static.	Changed.	Streaming.	Static.	Changed.	Static.	Changed.	Changed.	Changed.
hours.	*hours.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
17	17	5.0	3.0	0.5	5.0	6.5	2.0	7.0	0	2.0
24	7	0	0	0	2.0	1.0	0	0	0	0
41	17	Dead.	0	Dead.	0.5	1.5	0	4.0	0	1.0
48	7	...	Dead.	...	0.5	0.5	Dead.	1.5	Dead.	1.0
Total growth in 48 hours.....		...	...	...	8.0	9.5	...	12.5	...	4.0

The results presented in table 8, and especially the behavior of plant No. 9, suggested a possible importance of the rate of supply of oxygen, as determined by the frequency of change of the soil atmosphere, and this led to suspicion concerning the previously assumed unimportance of the traces of oxygen present in the two gases. Accordingly, experiments were arranged, using Canada field pea and the technique just described, but with nitrogen and helium from which the oxygen had been removed so far as was possible by treatment with the well-known solution of pyrogallol in an aqueous solution of potassium hydroxide. In order to obtain an additional check on the purity of the cylinder nitrogen, the nitrogen for a part of these experiments was made by treating atmospheric air with the alkaline pyrogallol. This nitrogen behaved exactly as did deoxygenated cylinder nitrogen. The treatment with alkaline pyrogallol was effected by allowing the air or other gas to stand for some hours in contact with the absorbing solution, usually with frequent shaking, and then passing the remaining gas slowly through two gas wash-bottles filled with fresh absorbing solution. The resulting gas contained no oxygen determinable by absorption in alkaline pyrogallol in the ordinary apparatus for gas analysis and produced no perceptible coloration in a fresh solution of alkaline pyrogallol. Parallel experiments were

arranged to test, as before, the effects of "static," "changed," and "streaming" atmospheres. The results are given in table 9, the arrangement of the data being the same as in table 8.

It will be noted that the data of table 9 are in contrast with those previously described, in that growth stopped promptly in both nitrogen and helium, this stoppage being followed in all cases by the early death of the root. The slight growth made in nitrogen during the initial 16-hour period can not be regarded as significant, since this may have occurred in the early hours of the experiment, during which the original oxygen had not been entirely swept out or the anaerobic conditions had not yet become effective.

TABLE 9.—*Growth of roots of Canada field pea in nitrogen and in helium, both free of oxygen.*

Exp.		Nitrogen.			Helium.		
		No. 10	No. 11	No. 12	No. 13	No. 14	No. 15
Time from beginning.	Time of growth period.	Static.	Changed.	Streaming.	Static.	Changed.	Streaming.
hours.	*hours.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
8	8	...	...	...	...	0	0
16	16	0.5	0.5	0.5	0	0	0
25	9	0	0	0	0	0	0
32	7	...	...	...	...	Dead	0
41	16	Dead	Dead	Dead	0	...	0
48	7	...	...	...	Dead	...	Dead

The effect of small quantities of oxygen in the gases was tested further by an experiment in which a single root was subjected to a streaming atmosphere of nitrogen with an oxygen-content decreasing by steps from 2.0 per cent to 0.5 per cent. The technique was arranged as before, the nitrogen-oxygen mixtures being made up by volume in metal gasometers and checked by analysis before using. Data are given in table 10.

The arrangement of table 10 is the same as that of previous tables, except that the observed amounts of growth are cumulated in the right-hand side of the table in order to compare the growth-rates in the soil atmosphere with different percentages of oxygen. The final, right-hand column gives the growth-rates, calculated to the uniform basis of milimeters growth per 24 hours. The same plant and culture tube were used throughout the experiment. It was first exposed for 17 hours to a slow stream of nitrogen containing 2.0 per cent oxygen. The main root grew 8.5 mm., or at the rate of 6.0 mm. per 24 hours. The gas stream was then changed to one containing 1.0 per cent oxygen and this was continued for 32 hours. Root-growth continued, but at the slower rate of 4.5 mm. per 24 hours. The gas stream was then changed to one of cylinder nitrogen containing 0.5 per cent oxygen

and this stream was continued for 88 hours. Growth gradually decreased, and had stopped altogether when one-half of the 88-hour period had elapsed. At the end of the 88 hours the culture tube was swept out with oxygen, the seal removed, and the soil allowed to remain open to the air. That the root had survived the 88-hour treatment with cylinder nitrogen was shown by an immediate resumption of growth, which was still in progress after 120 hours, the culture tube having been then under observation for over 10 days.

TABLE 10.—*Growth of roots of Canada field pea in nitrogen containing decreasing small percentages of oxygen.*

Time from beginning of experiment.	Observed growth since last observation.	Composition of gas, percentage.	Time in gas of given composition.	Total growth in this gas.	Growth per 24 hours.
hours.	*mm.*		*hrs.*	*mm.*	*mm.*
Start					
17	8.5	Nitrogen 98; oxygen 2.....	17	8.5	6.0
23	3.0				
41	3.0	Nitrogen 99; oxygen 1.....	32	3.0	4.5
49	0				
63	1.5				
94	0.5	Nitrogen 99.5; oxygen, 0.5 (cylinder nitrogen).....	88	2.0	0.5
113	0				
137	0				
161	6.0	Oxygen; then air.....	120	38.0	7.6
257	32.0				

Parallel with the cultures reported in table 9, another culture was treated with a static atmosphere of nitrogen containing 2.0 per cent oxygen. This culture showed continued root-growth for 53 hours, though at an average rate of but 2.8 mm. per 24 hours, which is substantially lower than that of the culture of table 9, which was exposed to streaming gas. A third culture, run later and not entirely parallel to that of table 9, was exposed to a static atmosphere of nitrogen with 1.0 per cent oxygen. There was slight growth during the first 12 hours, but none thereafter. However, the root was alive after 48 hours and resumed growth on being again supplied with air.

It is difficult to understand these results otherwise than as indicating the controlling rôle of the small percentages of oxygen in the gases. In nitrogen with 2.0 per cent of oxygen, growth continues normally, whether the atmosphere be streaming or static, at the temperatures used. With 1.0 per cent of oxygen, growth continues in a streaming atmosphere, but at a slower rate, and the one available experiment indicates that in a static atmosphere growth would stop altogether. In nitrogen, with 0.5 per cent of oxygen, the data of table 10 confirm the conclusion that growth will be seriously hampered and life endangered, even in a streaming atmosphere. With a static atmosphere death is still more certain. In nitrogen freed so far as possible from

oxygen, neither growth nor life is possible. The only discrepancy in the data is that between plant No. 3 of table 8, which died in a stream of cylinder nitrogen, and the plant of table 10, which did not die in the same gas. Perhaps the previous experience of the latter plant in nitrogen with 2.0 per cent, and 1.0 per cent may have in some fashion "acclimated" the root to partial deficiency of oxygen.

The results with helium are equally significant. From table 8 it is apparent that the 0.2 per cent of oxygen, which is present in the

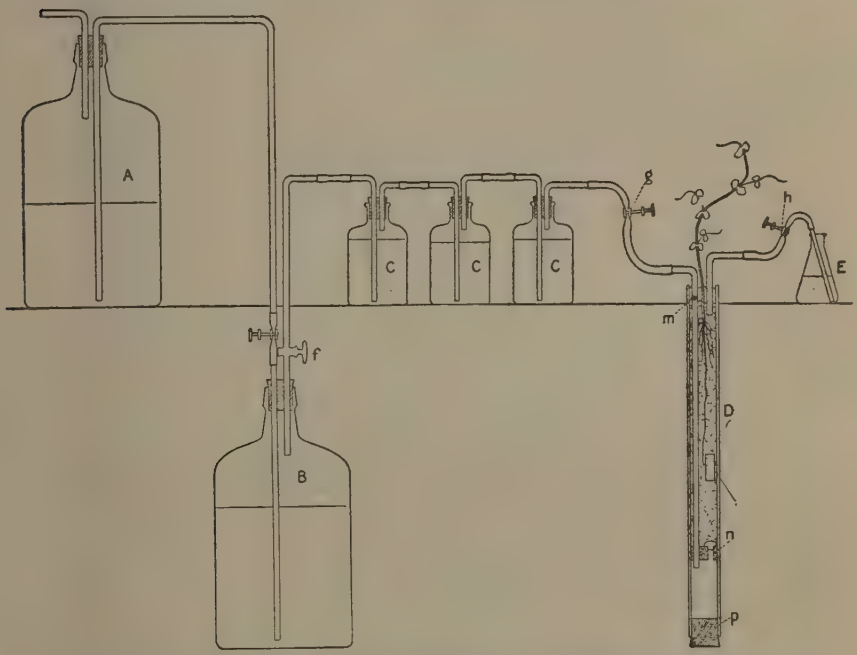

FIG. 10.—Arrangement of one form of apparatus used in anaerobic studies. An aspirator, with parts *A* and *B*, is connected by a chain of wash bottles, *C*, to the culture tube, *D*, ending in a flask containing water, *E*. The plant is sealed in tube with plasticine. The method used in marking growth of the root is shown, but without date, which, in actual practice, is also given.

cylinder helium, is sufficient to enable growth for 48 hours even in a static atmosphere. However, table 9 indicates that the entire removal of oxygen from the helium makes the gas fatal to the roots, whether or not the atmosphere is renewed. The difference between nitrogen and helium, which difference has been observed throughout these experiments, may be expressed, therefore, as a greater oxygen requirement of the root when the surrounding atmosphere is of nitrogen than when this atmosphere is of helium. The experiments which are significant in determining the quantitative value of this oxygen

requirement are those with streaming atmospheres. In these the gas concentrations are maintained constant, while in cultures in static atmospheres the respiration of the root continually decreases the oxygen. Accordingly, it may be said, on the basis of the data presented, that the minimum oxygen requirement for the growth of the roots of the Canada field pea, at any temperature, in helium is less than 0.2 per cent, while in nitrogen it is between 0.5 and 1.0 per cent.

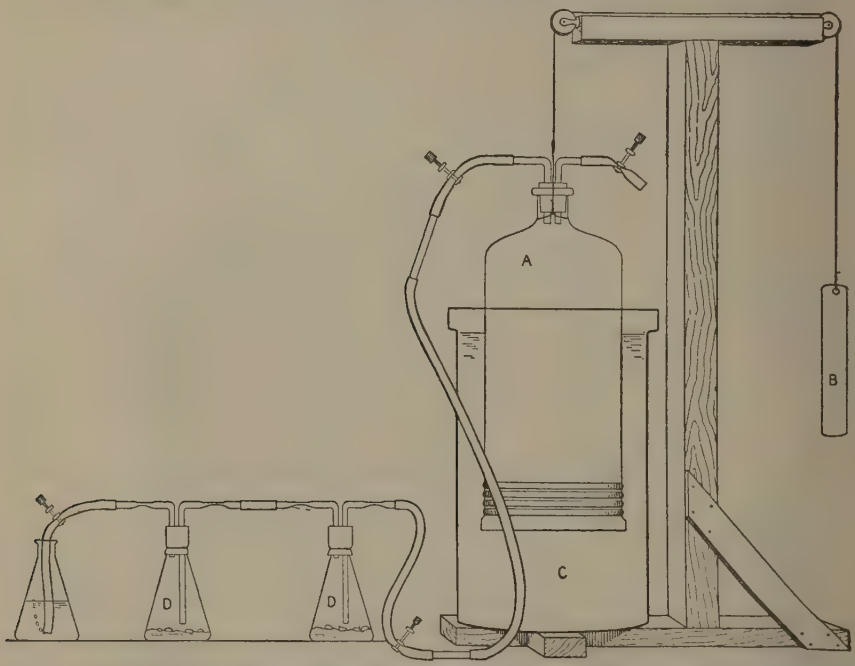

FIG. 11.—One kind of apparatus used in anaerobic studies. A bell-glass, *A*, weighted with lead pipe, is suspended by a cord with counterpoise, *B*, in a museum jar, *C*, partly filled with water. The bell-glass is provided with inlet and outlet tubes, of which the latter pass to culture flasks, *D*, *D*, and finally to an end flask with water. The experimental gaseous mixture is under slight pressure.

Two theoretical possibilities of error require notice. The first is the possible death of roots through lack of water rather than because of changes in aeration. This possibility is excluded, we believe, by the fact that cultures exactly like the ones used in the experiments have grown normally in culture tubes for as long as 6 weeks without watering. The record of table 10 covers 10 days of a similar plant. The second chance of error lies in the possible effect of carbon dioxid. A certain amount of this gas is formed in the culture tube by the respiration of the root and probably by bacteria. In a number of

analyses of gas from the tubes we have never found over 0.8 per cent. Usually the percentage has been too small for accurate determination. An experiment exactly like those with nitrogen, but in which the gas supplied to the culture tube was 70 per cent nitrogen, 20 per cent oxygen, and 10 per cent carbon dioxid, showed normal root-growth for 48 hours and no subsequent injury. Similar experiments with

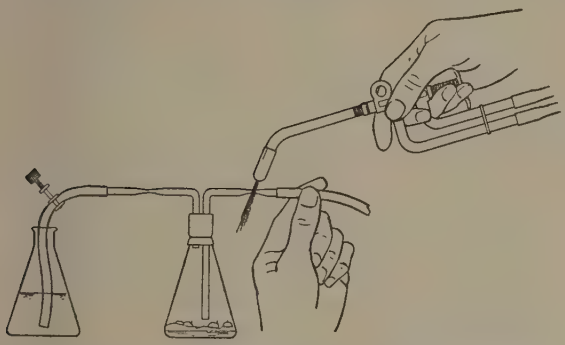

FIG. 12.—Method of sealing culture flask.

100 per cent carbon dioxid, and with a mixture of 75 per cent carbon dioxid and 25 per cent oxygen, showed stoppages of root-growth and injuries; but even 10 per cent of carbon dioxid is far above any concentration which could have occurred in the culture tubes during the experiments with nitrogen or with helium.

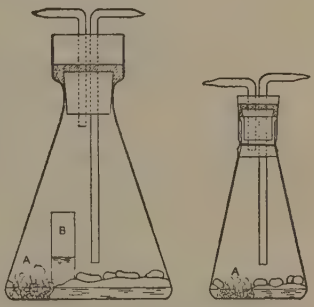

FIG. 13.—Sealed flasks containing ungerminated seeds of Canada field pea. For the purpose of removing possible traces of oxygen, steel wool, *A*, is included in each flask, and in one flask a small amount of sodium hydroxid, *B*, is kept to remove carbon dioxid.

In addition to the experiments on the root-growth of Canada field pea, tests were made of the germination of this species. 25 selected seeds were put in each of seven 200-c. c. Ehrlenmayer flasks containing a little cotton wetted with recently boiled distilled water. These flasks were closed with perforated rubber stoppers previously boiled in paraffin and equipped with two glass tubes for the passage of gases (figs. 11 to 15). Gas mixtures of known composition, consisting of nitrogen and helium with various percentages of oxygen, were then swept through the flasks until all air was removed, the glass tubes

were sealed off in the flame, and the cultures put in a constant-temperature cabinet at 24° to 25° C. and protected from direct light. A layer

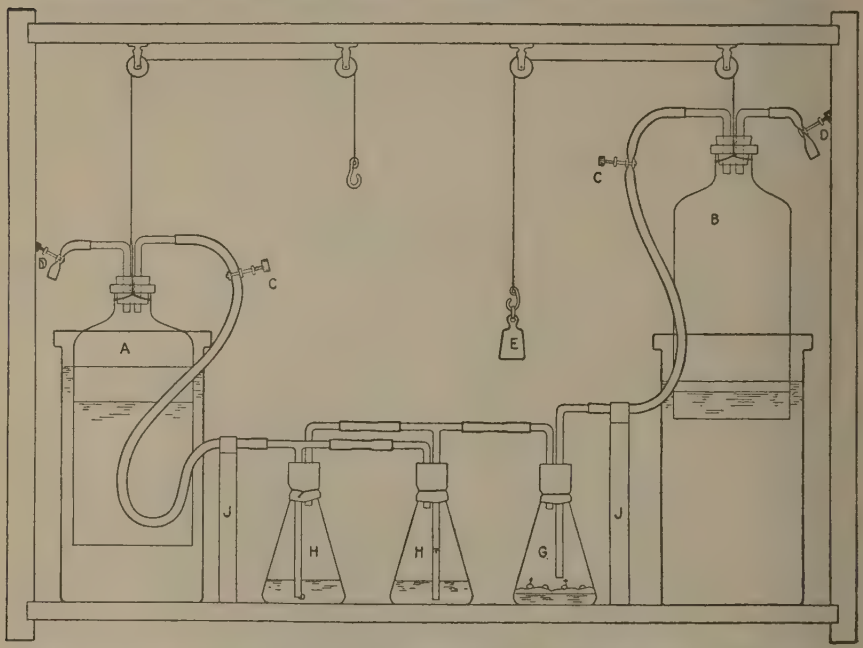

FIG. 14.—Apparatus used in anaerobic experiments in germinating seeds. Gasometers, of form shown in figure 11, are so connected with Ehrlenmayer flasks that a reciprocating movement of the contained gas is possible. In the figure the current is from *A* to *B*. By shifting the weight, *E*, to the hook attached to the cord of gasometer, *A*, the gas-flow is in the opposite direction. The circulation thus is closed.

of vaseline over the stopper and the rim of the flask served as an additional precaution for the prevention and immediate detection of leaks. The gas mixtures used were checked by duplicate analyses.

TABLE 11.—*Germination of Canada field pea in nitrogen and helium.*

Time in days from beginning of experiment.	Germinations.						
	Nitrogen, per cent O_2.			Helium, per cent O_2.			Air.
	0.5	1.4	6.3	0.2	1.4	6.5	
1	0	0	0	0	0	7	12
2	0	9	14	2	6	20	24
3	5	10	19	6	15	24	25
4	6	12	20	8	19	24	
5	Disinte-	14	20	9	19	24	
6	grated.	16	20	9	19	24	

The results are given in table 11, the figures being the number of visible germinations in each culture at the end of the given number of days. The control, marked "air," was closed with a perforated cork stopper, the perforation of which was open.

It is apparent that the greater oxygen requirement in nitrogen is again in evidence, the germination being clearly less prompt in the gas mixtures containing nitrogen than in those containing helium. On the fifth day the seeds in the cylinder nitrogen were entirely disintegrated by a gaseous decay, the nature of which was not investigated. On the sixth day the stoppers were removed and the cultures exposed to air. No further germinations occurred and there was no growth of either radicle or shoot from any of the germinated seeds.

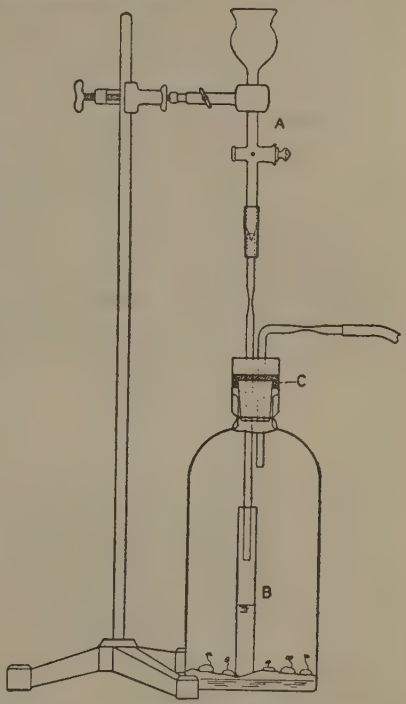

FIG. 15.—Apparatus for the introduction of oxygen-free water into anaerobic cultures. The seeds are placed at the bottom of the container on dry cotton. Freshly boiled water is introduced into the internal test-tube through the glass tube and funnel. After the exhaustion of the container and the supply to it of the desired gas atmosphere the test tube is tilted and the water run out to saturate the cotton. *C* indicates a mercury seal around the stopper.

EXPERIMENTS WITH MAIZE.

In experiments with *Zea mays*, the glass culture tubes were employed according to a technique the same in all particulars as that described for Canada field pea. Four experiments were made with cylinder nitrogen and four with cylinder helium, all at 23° to 24°C. In each case 2 of the experiments were with static atmospheres and in 2 the gases were changed. In addition, 2 experiments with nitrogen and 2 with helium were made at 27° to 28° C., all with static atmospheres. In all cases root-growth stopped during the initial period of 8 to 16 hours and was not resumed in 48 hours. In the case of the 8 experiments at 23° to 24° C. the gas was swept out by oxygen at the end of 48 hours. All roots recovered and began growth. In the case of the 4 experiments at 27° to 28° C. the application of the anae-

robic gas was continued for 96 hours. There was no growth, and root growth was not resumed in 48 hours after the roots had been supplied with oxygen.

While these experiments do not permit firm conclusions as to the behavior of the roots of maize under anaerobic conditions, it is obvious that there is no indication of a difference between nitrogen and helium such as was observed with potato and Canada field pea. No experiments have yet been made on maize with higher or lower percentages of oxygen than were present in the cylinder gases.

EXPERIMENTS WITH THE STIGMATAL MOVEMENT OF DIPLACUS.

The flower of *Diplacus glutinosus*, which grows in profusion in the neighborhood of the Coastal Laboratory, possesses a bifurcated stigma which is extremely sensitive to mechanical stimuli. The normal open position of the stigma is with the two lips or leaves extended in apposition, like the covers of an open book (fig. 16). The upper

FIG. 16.—Figures illustrating stigmatal movement of *Diplacus glutinosus*. The usual open position appears in *A*, and complete closure of the lobes of the stigma following a stimulus in *B*.

surfaces of these lips are plentifully supplied with papillæ, and when these papillæ are touched by a rough object the lips of the stigma come together, as the covers of a book when it is closed. Many observations of this reaction, both in flowers still attached to the plant and in cut flowers in water, have shown that the times of perception and reaction are remarkably independent of external conditions. The movement begins in from 5 to 20 seconds after the stigma is touched and is complete in from 30 to 60 seconds. These times are not varied outside of the given limits by light intensity from full sunlight to darkness, nor by temperature between 10° and 40° C., nor by the humidity of the air between full saturation at 40° C. and the aqueous vapor-pressure over concentrated sulphuric acid at 10° C. The times are not perceptibly different for flowers attached to the plant and for flowers which have been cut off and kept with their stems in water for as long as 3 weeks. During the life of the

flower between its first opening and the visible wilting of the petals the reaction-time of the stigma remains the same. Indeed, normal reactions can sometimes be obtained from the stigma of immature flowers or of flowers the petals of which are already dead and wilted. Some minutes after closure following mechanical stimulation the stigma of a healthy flower will reopen and is again sensitive to stimulus. This closure and opening may be repeated many times, without sensible effect on the reaction times. In the case of one cut flower we obtained normal response to 31 successive mechanical stimulations, after which the stigma remained closed. The time required for re-

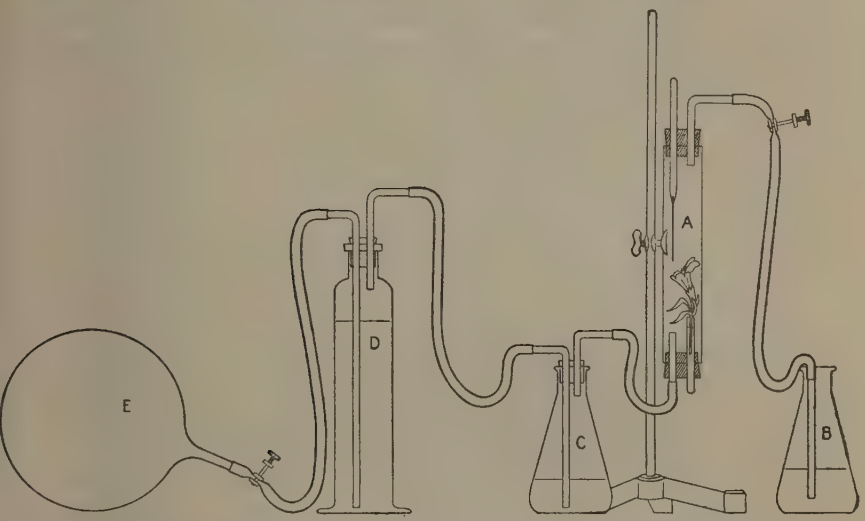

FIG. 17.—Apparatus assembly for investigations on stigmatal movement in *Diplacus*, in relation to stimuli of various kinds, including different gaseous mixtures. The experimental gas is forced by the contraction of the rubber container *E*, through wash bottles *D* and *C*, to the chamber *A*, containing the flower and out through a final wash bottle with water.

opening after closure is not so invariable as are the times of perception and reaction. It appears to depend, however, more upon the health of the flower than upon any external condition, except possibly light. In our observations this time of reopening has varied between 10 minutes and 2 hours.

In agreement with the results of Correns on the reactions of the stigma of *Mimulus* and the stamens of *Berberis*,[1] we have found that the stigma of *Diplacus* is sensitive of deficient aeration. The apparatus employed was similar to that described by Correns (figs. 17, 18), and consisted of a glass tube 15 cm. long by 3 cm. internal diameter, held

[1] Flora, 75: pp. 99–115. 1892.

vertically in a metal clasp and closed at the ends with 2-hole rubber stoppers. The holes of the lower stoppers served respectively for the gas-inlet tube and to hold a short glass tube, sealed at the lower end and filled with water. Into the upper open end of this tube the stem of the flower was inserted, the flower being thus held upright inside the larger glass tube which served as receptacle. The holes of the upper rubber stopper of the receptacle held, respectively, the gas-outlet tube and a glass rod bearing at its lower extremity, inside the receptacle, a copper wire. By virtue of the elasticity of the upper rubber stopper, this copper wire, when properly adjusted in advance, could be brought in contact with the stigma of the flower and served to produce mechanical stimulus if desired. Exposure to the desired gas was effected by passing a fairly rapid stream of the gas through the receptacle.

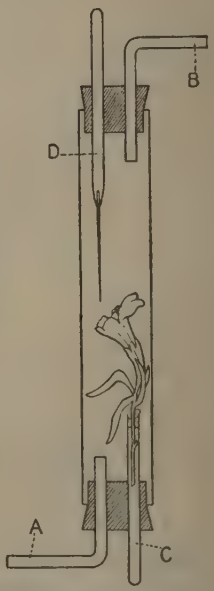

FIG. 18.—Construction details of gas chamber used in experiments on *Diplacus*. *A* and *B*, intake and outlet tubes. *C*, glass receptacle with water and stoppered with cotton, for holding the flower. *D*, glass or glass and metal rod, which can be moved laterally, for mechanical stimulation of the stigma.

Preliminary experiments with this apparatus having established that the stigma closed spontaneously, without mechanical stimulus, after from 3 to 10 minutes in nitrogen or helium, a suite of experiments was arranged with nitrogen and helium containing known percentages of oxygen. It had also been determined, of course, that the stigma would not close spontaneously in this apparatus merely in a stream of air, whether dry or moist, and that the normal reactivity to mechanical stimuli was retained by stigmas which had been several hours in air inside the apparatus.

The experiment with nitrogen-oxygen mixtures began with a mixture determined by duplicate analysis to contain 12.7 per cent of oxygen by volume. Passage of this gas for 20 minutes produced no reaction of the stigma. The gas stream was then changed to one containing 6.3 per cent of oxygen, which also produced no reaction in 20 minutes. The final change, to a composition of 1.4 per cent of oxygen in nitrogen, caused closure of the stigma beginning at 3.5 minutes and complete in 6 minutes. A repeated test, in which nitrogen containing 1.4 per cent of oxygen was passed over another flower, showed closure of the stigma beginning at 6 minutes and complete in 14 minutes. These tests were all run in sunlight at 28° to 31° C.

A similar series with helium began at an oxygen-content of 10.0 per cent. This oxygen-content was decreased successively to 6.5 per cent, 3.0 per cent, and 1.4 per cent, without any of these gas mixtures producing reaction. Each mixture was passed through the receptacle for 15 to 18 minutes. The final reduction to the cylinder helium, containing 0.2 per cent of oxygen, caused reaction beginning at 6 minutes and complete in 15 minutes. A repeated test on another flower began with 3.0 per cent of oxygen in the helium and again showed no reaction at this composition or with 1.4 per cent of oxygen. Final reduction to 0.2 per cent of oxygen again caused reaction, beginning this time at 6.5 minutes and complete in 15 minutes. As before, the compositions of the gases were determined by duplicate analyses and the tests were run in sunlight at 28° to 31° C.

In our opinion, the significant feature of these results is the fact that closure of the stigma occurred in nitrogen with 1.4 per cent of oxygen as well as in the cylinder nitrogen, but did not occur in 15 minutes in helium with the same percentage of oxygen. As in the case of the root experiments on the Canada field pea, there is a difference in the percentage of oxygen required for the reaction, depending upon whether the surrounding gas is nitrogen or helium. What may be designated the "oxygen requirement" under these conditions is between 1.4 per cent and 6.5 per cent in nitrogen and is between 0.2 per cent and 1.4 per cent in helium. As will be discussed in a later section of this paper, these results, as well as those previously presented, are entirely in harmony with the hypothesis that the difference between the gases lies in the less rapid diffusion of oxygen through nitrogen.

Two experiments each on the reaction of the stigma of *Diplacus* in nitrogen and helium at lower temperatures, 15° to 16° C., showed reactions in nitrogen beginning at 17 and 18 minutes respectively and complete in 20 minutes, and reactions in helium beginning at 17 and 22 minutes, respectively, and complete in 22 and 27 minutes. Two other experiments were run with flowers previously kept for 15 minutes in oxygen instead of in air. One of these in cylinder

nitrogen showed a reaction beginning in 11 minutes and complete in 14 minutes. The other in cylinder helium showed a reaction beginning in 6 minutes and complete in 10 minutes. Conclusions based upon differences in times of reaction must necessarily be statistical, and the available observations are insufficient to render such conclusions possible. However, the data of this paragraph, so far as they have weight at all, are in accord with the diffusion hypothesis. In experiments with cylinder nitrogen and cylinder helium at 28° to 31° C., the times of reaction varied as widely between individuals tested in the same gas as between the averages of tests in the two gases.

Other experiments on the reaction of *Diplacus* in carbon dioxid, ethylene, acetylene, illuminating gas, hydrochloric acid, nitric oxide, sulphur dioxid, chlorine, iodine vapor, ammonia, ether vapor, and the vapor of petroleum ether are of no interest in the present connection, but offered no facts not exactly harmonious with the diffusion hypothesis.

EXPERIMENTS WITH OTHER SPECIES.

Some incomplete experiments with other species may be described briefly.

A single test of the growth of the main root of sweet pea, *Lathyrus odoratus*, in cylinder nitrogen, and two tests of its growth in cylinder helium were made, using the glass-tube method described for Canada field pea. Growth stopped at once in nitrogen. With streaming helium, growth continued normally for 3 days. With static helium, growth stopped within 48 hours.

A similar experiment was made with a hybrid potato, *Solanum fendleri* × *S. tuberosum*, F_1,[1] but in this case the growth of the shoot was measured instead of that of the root. With the roots in cylinder nitrogen the shoot did not grow; with the roots in cylinder helium it grew 3.4 mm. in 24 hours.

Germination tests of rice, *Oryza sativa*, in cylinder nitrogen and in cylinder helium were made, using the technique described for germination tests of Canada field pea. The seed used was kindly supplied to us by Dr. W. W. Mackie, of the University of California. Germinations began in the helium on the fourth day; in the nitrogen on the fifth day. On the sixth day 16 seeds out of 50 had germinated in the nitrogen and 28 out of 50 in the helium. On the eighth day the respective numbers were 20 and 30, and on the twelfth day, when the experiment was stopped, 49 seeds had germinated in the nitrogen and 48 in the helium. A third culture set up later with helium which had been deoxygenated by alkaline pyrogallol showed germinations

[1] MacDougal. Carnegie Inst. Wash. Year Book for 1917, p. 98; Year Book for 1918, pp. 87–88.

almost exactly parallel to those of the culture in cylinder helium. The ability of rice to germinate under anaerobic conditions is well known, and the differences between the cultures in nitrogen and in helium are not wide enough to be importantly significant if the experiment stood alone. It is noteworthy, however, that the difference is in the same direction as in the other cases. It was noticed also that the growth of the shoot and the formation of chlorophyll in it were unmistakably faster in the cultures in helium than in that in nitrogen. On the eighth day the culture in nitrogen had 10 green shoots 5 to 20 mm. long; the culture in cylinder helium had 21 green shoots 5 to 30 mm. long.

Similar germination tests with lettuce, *Latuca sativa*, showed no germination in 4 days, either in nitrogen or in helium, although a control in air showed a high percentage of germination on the second day. On the fourth day the nitrogen and helium cultures were opened to the air. Three days thereafter there were 43 germinated seeds in the culture which had been in nitrogen and 63 in the one which had been in helium. Each culture contained approximately 300 seeds. Apparently the injury by 4 days' exposure to nitrogen is greater than that by exposure to helium for an equal period. As before, the gases were as drawn from the cylinders.

Seedlings of sunflower, *Helianthus annuus*, were grown for 4 days, in the dark, on wet cotton, in flasks similar to those described for the germination tests on Canada field pea, but open to the air. On the fourth day the seedlings were 2 to 8 cm. high and were etiolated. In one culture the air was replaced with cylinder nitrogen and the culture sealed. A second culture was prepared similarly with cylinder helium. A third was left open to the air. All were so placed as to be exposed to light on one side only. The three cultures were similar, so far as could be determined by visual observation. 24 hours after sealing there had been no growth in the nitrogen culture, 4 out of the 10 seedlings of this culture were wilted and had brownish discolorations on the hypocotyl, and there was no evidence of the formation of chlorophyll in any seedling. In the helium culture there had been growth of 1 or 2 cm., there were no visible symptoms of injury, and a distinct formation of chlorophyll was noticeable. There were some doubtful indications of heliotropic curvature in the helium culture, but none in the nitrogen culture. The control in air showed growth of 2 to 3 cm., marked greening of all seedlings, and distinct heliotropic curvature. After 24 hours in the anaerobic gases the seedlings were again exposed to air. All of the seedlings from the nitrogen culture died; 4 out of 11 from the helium culture survived, though with distinct evidence of injury. It is interesting, though without apparent significance for the present inquiry, that the injury to the seedlings both in nitrogen and in helium was entirely to the hypocotyl and to the lateral

roots. These turned brown and immediately began to decay. There was no apparent injury to the cotyledons or to the main root.

Seedlings of mustard, *Sinapis alba*, grown in the same manner as the seedlings of sunflower and similarly exposed to cylinder nitrogen and cylinder helium for 24 hours, showed no perceptible differences between the two cultures. In both gases there was a cessation of growth and inhibition of the formation of chlorophyll and of heliotropic curvature. This is in agreement with the results of Correns,[1] who found that the percentage of oxygen necessary for the growth of mustard seedlings was greater than that necessary for sunflower seedlings.

Mature plants of *Oxalis acetosella* were placed in closed jars containing cylinder nitrogen and cylinder helium respectively. A third plant in a similar jar, not sealed, served as control. After 3 hours the plant in nitrogen showed marked closure of the leaves. No closure was perceptible in helium or in the control. All plants were exposed to diffuse light. This experiment was twice repeated with similar results. On the second repetition the plants were left in the gases for 5 days and were in the dark after the first 12 hours. At the end of the 5 days the plant in nitrogen showed nearly complete closure of leaves. The plant in helium showed distinct closure, but less than that of the plant in nitrogen. The control was open. On removal from the jars, both plants which had been under anaerobic conditions lost a number of leaves by an injury the first symptom of which was a chlorosis of the leaf margins. This was about equally severe in the two plants. The control did not show it.

Young plants of an acacia, probably *Cassia* sp., 8 to 10 cm. high and growing in soil, were placed in jars in diffuse light and exposed respectively to cylinder nitrogen, cylinder helium, and air. The experiment was set up at $9^h\ 15^m$ a. m.; at $6^h\ 30^m$ p. m. the usual diurnal movement of the leaves toward the night position was complete in the plant in air and nearly complete in the plant in helium. In the plant in nitrogen the movement was very slight, though distinctly perceptible. At $8^h\ 30^m$ the following morning the plants in helium and in air were normally open, the plant in nitrogen was about one-half closed. All were open normally at 11 a. m. All three were then removed from the jars and shaken violently for several minutes. In the plants from helium and from air the leaves closed at once. In the plants from nitrogen there was no closure of the leaves until night. Apparently anaerobic conditions tend to retard the nocturnal closure and the reaction to mechanical stimulus, and this interference is more marked in cylinder nitrogen than in cylinder helium.

Four samples, each of 75 grams, of air-dry seed of soy bean, *Glycine hispida*, were sterilized with a dilute solution of potassium hypochlo-

[1] Flora, 75: 131–142. 1892.

rite, according to the method of Duggar and Clark, placed in sterile glass jars equipped with gas outlets and wetted with sterile distilled water. The jars were then filled, respectively, with (1) cylinder nitrogen, (2) cylinder helium, (3) a mixture of equal parts of nitrogen and helium, and (4) air. All were sealed and placed in the constant-temperature cabinet at 24° to 25° C. The course of the respiration was followed by manometers attached to the jars and its amount determined, finally, by analysis of the inclosed gas. After 48 hours the jar with nitrogen contained 34.0 per cent carbon dioxid, the jar with helium contained 37.6 per cent, the jar with the nitrogen-helium mixture contained 36.3 per cent, and the jar with air contained 33.0 per cent. These percentages are by analysis, but were in agreement with the manometer readings. Among the anaerobic preparations the highest respiration was in helium and the lowest in nitrogen, the one in the mixed gases being intermediate. The preparations were kept under observation for 3 days longer without any bacterial action being perceptible.

A similar respiration experiment was made with unripe tomatoes, the 4 samples containing each 3 or 4 fruits of approximately equal total surface, though of weights varying between 95 and 150 grams per sample. The fruits were sterilized by the hypochlorite solution, as before. After 48 hours at 24° to 25° C. the percentage of carbon dioxid in the preparation in cylinder nitrogen was 15.9 per cent, in cylinder helium it was 19.6 per cent, in the nitrogen-helium mixture it was 17.4 per cent, and in air it was 14.0 per cent. The results are parallel, therefore, with those on the respiration of the germinating soy beans.

Tubers of the potato hybrid already mentioned were kept for 11 days in sealed glass jars filled respectively with cylinder nitrogen, cylinder helium, and oxygen. All gases were kept moist. It was known that tubers of this hybrid are exceptionally resistant to the ordinary forms of decay attacking the potato. Of the tubers in helium, 3 out of 12 were decayed and moldy. Of those in oxygen, 4 out of 11 were similarly destroyed. Of 12 tubers in nitrogen, none was affected. Some of the tubers in oxygen and in helium had put out new shoots, but these had grown only a few millimeters. If there was any difference, the growth was greater in helium than in oxygen. None of the tubers in nitrogen developed shoots. In this particular experiment the precautions against inward leaks were not so complete as usual, and the results are not entirely free from suspicion on this ground.

While no great weight can be given, individually, to any of the experiments described in this section of this paper, they do have when taken together, a certain cumulative force. It will be noticed

that a difference between the cylinder nitrogen and the cylinder helium was observed very generally, and that, in cases in which the conditions permit analysis of the phenomena, this difference is always in the same direction. The vital activity is more promptly or severely disturbed in nitrogen than it is in helium.

TESTS FOR IMPURITIES IN THE GASES.

It has already been noted that both gases, as procured, contained small amounts of oxygen. The amounts present were determined by us, using alkaline pyrogallol in the standard Orsat apparatus for gas analysis. The deficiencies of this apparatus were known to us, but no equipment of greater accuracy was at hand or procurable within a reasonable time. Every effort was made to obviate the usual errors of the apparatus. Important analyses were made at room temperatures constant within 1° C. and which had been so constant for several hours before the analysis, this being easily arranged under the equable summer temperatures of the Coastal Laboratory. Fresh reagents were employed and every care was taken with details of manipulation. Most of the analyses were made over water previously saturated with the gas in question. Four analyses of helium over mercury were confirmatory, but probably no more accurate, since the strongest rubber tube available to us suffered a certain deformation when filled with mercury. In gases known to contain no carbon dioxid, it was possible to use two of the absorption pipettes of the apparatus for gas storage during our analysis and thus to work with 200 c. c. of gas instead of 100 c. c., concentrating the entire absorption in one measurement. The gas burette could be read to 0.05 c. c., but it is doubtful if the determinations are accurate beyond 0.1 c. c. Duplicate determinations of oxygen or of carbon dioxid, when small amounts only were present in the gas sample, usually agreed to 0.1 per cent, but variations as wide as 0.2 per cent were sometimes observed. Samples taken as supposed duplicates frequently varied more widely than this, but such variations are to be expected from errors in sampling, especially with oxygen, a slight leakage from the atmosphere being always a possibility.

In the determination of the percentages of oxygen in the cylinder gases, all of the known and possible precautions were employed, and errors of sampling were eliminated, so far as possible, by direct attachment of the cylinders to the apparatus, proper safety devices alone being interposed, and by sweeping out the entire apparatus and gas-train thoroughly with the cylinder gas before the analysis was begun. Determinations of oxygen in the nitrogen, made in this way, gave percentages varying between 0.4 and 0.6. Nearly all of the determinations were either 0.50, 0.55, or 0.60 per cent. Similar determinations on the helium have percentages between 0.1 and 0.2. A few

were below 0.1 and not surely measurable. None was above 0.2 per cent. That the absorption was really due to oxygen in both cases was established by the characteristic color reaction with a fresh solution of alkaline pyrogallol. We believe it may be concluded, safely, that the nitrogen used by us contained between 0.5 and 0.6 per cent of oxygen and the helium contained between 0.1 and 0.2 per cent. Since the biological results described indicate a lesser activity of the oxygen in the nitrogen, we have chosen the oxygen percentages used throughout this discussion on opposite sides of the limits found by analysis. The nitrogen is considered to contain 0.5 per cent, whereas it probably contains slightly more than this. The percentage in the helium is taken as 0.2 per cent, whereas it is almost certainly less than this. Accordingly, any possible error in the chemical determinations will probably be in a direction opposite to the biological differences discovered, and can not be used to explain these differences, unless, of course, the errors in our analysis are much greater than we think. In the light of the general consistency of all the biological results, especially those with still higher percentages of oxygen, this does not seem probable.

In addition to the trace of oxygen, the helium was known to contain approximately 4.0 per cent of nitrogen. This was not determined by us. Nor was any effort made to determine argon in either gas. Neither gas contained any carbon dioxid determinable by absorption in solution of potassium hydroxid or any carbon monoxid determinable by absorption in an acid solution of cuprous chlorid. Tests for hydrocarbons were made by exposing the gas, with added oxygen, to a heated platinum wire. In neither the nitrogen nor the helium was there any visible increase in the temperature of the wire, as indicated by its visible radiation, and in neither case was any gas absorbable in potassium hydroxid produced by 30 minutes' exposure to the heated wire. It is believed that anything over 0.2 per cent of methane could have been detected in this way. Ethane, ethylene, or higher hydrocarbons, and the combustible organic compounds, such as alcohol or ether, would have been detected even in lower percentages. Hydrogen would also be detected, although probably only in somewhat larger percentage. After a stream of the nitrogen or the helium had been passed for an hour through recently boiled distilled water, the colors given by this water with indicators were not different from those of the same water not treated with the gas. The indicators employed were phenophthalein, methyl-orange, methyl-red, brom-phenol-blue, brom-cresol-purple, brom-thymol-blue, and phenol-red. This appears to exclude any gases capable of imparting an acid or alkaline reaction to water. Filter-paper moistened with a weakly acid solution of lead acetate showed no coloration after 24 hours' exposure to either gas. This excludes hydrogen sulphid and the

complex sulphur compounds which yield it. Paper moistened with a dilute solution of potassium iodid and starch was similarly unaffected by the gases, as was also a piece of light-red cloth moistened with dilute potassium hydroxide. This excludes chlorine and gases capable of yielding it, as, for instance, phosgene or the oxids of chlorine. So far as we are aware, the only gases or usual vapors which would not be detected by these chemical tests are arsine and stibine. Even these would probably manifest themselves by an effect on the reaction of water toward some of the indicators used. It is possible, also, that small fractions of 1 per cent of such gases as carbon monoxid, hydrogen, or hydrocarbons might be present and have escaped the tests. Of these only ethylene and perhaps acetylene are known to be biologically active in such small concentration. Tests for these were made, therefore, by biological methods.

Since the reaction of opening flower-buds of carnation is among the most sensitive tests for ethylene and related toxic gases,[1] 4 sets of 5 buds each, in selected stages from those nearly open to those just beginning to open, were exposed for three days, respectively, to nitrogen with 20 per cent oxygen, helium with 20 per cent oxygen, air, and air with approximately 1 per cent of ethylene. The buds in nitrogen, helium, and air opened normally. Those in air with ethylene showed the characteristic injuries described by Crocker and Knight and others. A similar test with opening flower-buds of the garden geranium, *Pelargonium zonale*, resulted similarly. The buds exposed to ethylene opened partially and lost all petals. Those in nitrogen, helium, and air opened normally. Similar results were obtained with flowering plants of *Impatiens* sp., probably *sultani*. In air with ethylene, the flowers fell at once and the plant was permanently injured. In nitrogen, helium, and air, conditions remained normal. With young plants of carnation, not in bloom, neither nitrogen, helium, nor air had any effect, but neither was any effect perceptible in air with 1 per cent of ethylene. All of these experiments were conducted at room temperatures, 15° to 25° C., and in the light, the plants being in sealed glass jars or under bell-glasses sealed to glass plates with plasticine. All experiments ran for 3 days or more, and all gases contained approximately 20 per cent of oxygen. A single experiment with a mature plant of sweet pea kept for 5 days in helium, with oxygen, as usual, showed no perceptible effect.

The effect of hydrocarbons on young seedlings of pea having been described by Neliubov[2] as characteristically toxic, experiments similar to those just described were made with seedlings of Canada field pea growing in soil and 1 or 2 cm. high. Five seedlings, all growing in the

[1] W. Crocker, and L. I. Knight. Plant World 12: 83–88, 1909; Bot. Gaz., 55: 337–371 1913. S. L. Doubt, Bot. Gaz. 63: 209–224, 1917.

[2] Cited from V. I. Palladin. Plant physiology, trans. by B. E. Livingston, p. 231, 1918.

same small clay pot, were placed in each of the four gases—nitrogen with 20 per cent oxygen, helium with 20 per cent oxygen, air, and air with approximately 1 per cent ethylene. The cultures were in sealed glass jars kept in the constant-temperature cabinet at 24° to 25° C. and in diffuse light. After the 3 days the cultures in nitrogen, helium, and air were indistinguishable in condition and had average heights of 8.7, 8.7 and 9.2 cm. respectively. Heliotropism and geotropism were normal. The seedlings exposed to ethylene were clearly injured and had an average height of only 3.6 cm. The disturbance of geotropism implied by Palladin's citation of Neliubov was not observed. A precisely similar experiment with young seedlings of maize, *Zea mays*, led to equivalent results, except that the injurious effects of ethylene were not so clearly evident.

In connection with these biological indications against any toxic impurity in the gases, there should be recalled the experiments reported previously in this paper on the growth of roots of Canada field pea in nitrogen and in helium containing oxygen, the observation that the stigma of *Diplacus* showed no reaction to either gas when sufficient oxygen was present, and the fact that no differential effect between the two gases was observed when the oxygen was entirely removed.

A final test for toxic impurity in the helium, especially for carbon monoxide, was made with a living canary-bird, this bird being especially sensitive to that poison. The bird was placed in an atmosphere of helium with 20 per cent of oxygen for 1 hour and 30 minutes without any injurious effect being observed. During this period the atmosphere was four times renewed. The bird was then placed for 20 minutes in an atmosphere of helium with 10 per cent of oxygen. After 15 minutes the bird showed some signs of respiratory distress, but no sign of the collapse characteristic of carbon-monoxid poisoning. On removal to the air the bird appeared to be entirely normal and developed no perceptible after-effects. One of us breathed helium with approximately 20 per cent of oxygen for about 20 minutes without any feeling of discomfort or subsequent headache.

THE RÔLE OF GAS DIFFUSION.

No one can be more conscious than are the writers of the fact that the experiments herein reported fall short of what is desirable in precision and completeness. It is believed, however, that a difference in the behavior of small amounts of oxygen contained in atmospheres of nitrogen and of helium may be considered as established, and that this difference implies the importance of a factor previously insufficiently considered in investigations of anaerobiosis—the factor of gas diffusion. The experimentation reported apparently establishes a

somewhat general difference in the response of plant materials to nitrogen containing 0.5 per cent of oxygen and to helium containing 0.2 per cent of oxygen. The difference is in the direction reverse to that which would be expected on the ground merely of oxygen-content. The nitrogen contains more oxygen, but is less favorable to plant activities. It is especially significant that the direction of the difference between the two gases is the same in all cases. This paper reports all of our experiments in which nitrogen and helium were compared, no experiment being omitted except two with Canada field pea in helium, which were lost by breakage of the apparatus. Every one of these experiments in which there is any difference at all between the gases shows the difference to be in the same direction. It is nearly impossible that this consistency can have been accidental.

The experiments on the germination and root-growth of Canada field pea and on the stigmatal movement of *Diplacus* render it probable that the difference between the gases lies in the fact that the oxygen required by plant tissues is greater when the diluting gas is nitrogen than when it is helium. If we knew nothing further, this lesser oxygen requirement in helium might be ascribed to a specific beneficial effect of helium or of some impurity in it, or to a specific injurious effect of nitrogen or of some impurity in it. A specific effect of helium itself is extremely improbable because of the complete chemical inertness of this element. In spite of the entirely negative results of our chemical and biological tests, the presence of a small percentage of some impurity in one or the other gas can not be considered entirely impossible. Nor is it permissible to conclude that a specific effect of nitrogen is entirely impossible. However, both of these theoretical explanations are highly improbable. It is difficult to imagine that nitrogen, or any conceivable gaseous impurity, would be active toward protoplasm only when certain small partial pressures of oxygen were present and would lose this activity entirely when the oxygen-supply was greater or when there was no oxygen at all. It is more reasonable to seek an explanation in the known properties of nitrogen and of helium, especially any properties which would affect the behavior of small amounts of oxygen present in the other gases.

The only physical difference between nitrogen and helium which is known to be effective in this manner is that between the rates of diffusion of other gases, including oxygen, in them. The mutual diffusion of gases depends upon the kinetic properties of the molecule. At a given temperature, pressure, and concentration gradient, the rate of diffusion is expressed by a coefficient which gives the amount of the diffusing gas passing a unit of cross-section area per unit of time. This diffusion coefficient differs for each pair of gases, but is nearly constant for a given pair, regardless of the relative amounts of the two gases. For the diffusion of oxygen into nitrogen this diffusion coeffi-

cient has been found to be between 0.171 and 0.179.[1] This represents the mass of oxygen diffusing in 1 second across an area of 1 sq. cm. by virtue of a concentration gradient equal to a difference of 1 gram per cubic centimeter for each centimeter of distance. The total pressure of the gases is 760 mm. and the temperature is 0° C.

The similar diffusion coefficient for oxygen into helium does not appear to have been measured, but may be calculated by means of a formula based upon the diffusion theories of Stefan and Maxwell. This formula is given by Jeans,[2] as,

$$D = \frac{1}{3\pi n S^2}\sqrt{C_1^2 + C_2^2}$$

In this formula, D is the coefficient of diffusion, n is the number of molecules of a gas in 1 c. c. at 0° C. and 760 mm. pressure, S is the arithmetical mean of the diameters of the molecules of the diffusing gases in centimeters, and C_1 and C_2 are the respective mean molecular velocities of the two gases in centimeters per second. The value of n is determinable from the constant of electrolysis and the electronic charge. Using Millikan's latest value for the latter, 4.774×10^{-10} electrostatic units,[3] the value of n is 2.705×10^{19}. This differs only slightly from the value of 2.75×10^{19}, employed by Jeans. The molecular diameters of the two gases, necessary to determine S, may be calculated in several ways. The best values for helium are probably those derived from the viscosity and the thermal conductivity and are given by Jeans[4] as 2.16×10^{-8} and 2.28×10^{-8}, respectively. The mean of these figures, or 2.22×10^{-8}, may be considered a probable value. The corresponding values for oxygen, also given by Jeans, are 3.64×10^{-8} and 3.60×10^{-8}, the mean being 3.62×10^{-8}. Adopting these two means, the value of S for the oxygen-helium pair is the arithmetical mean of the two or 2.92×10^{-8}. The values of C_1 and C_2 are given by Jeans[5] as $1{,}208 \times 10^2$ for helium and 425×10^2 for oxygen. Substituting these several figures in the formula given one obtains for the coefficient of diffusion of oxygen in helium the value 0.589. That this method of obtaining the diffusion coefficient by calculation is sufficiently accurate for the purposes of this discussion is indicated by the comparisons of table 12, in which diffusion coefficients calculated by the above formula are compared with those obtained experimentally.[6] The direction of diffusion is neglected, since the coefficient for a given pair of gases is nearly independent of which gas is the one which is diffusing into the other. The data for S, C_1,

[1] Smithsonian Physical Tables, sixth edition, p. 140. 1916.

[2] Dynamical theory of gases, 2d ed., p. 332. 1916. Chapter 13 of this work contains full details of the calculations used in this section of this paper.

[3] The Electron, p. 119, 1917. [4] *Loc. cit.*, p. 341. [5] *Loc. cit.*, p. 131.

[6] Experimental data are from Jeans, *loc. cit.*; Smithsonian Physical Tables, *loc. cit.*; and Kaye and Laby, Physical and Chemical Constants, p. 35, 1911.

and C_2 used in calculating the coefficients from the formula are those given by Kaye and Laby and by Jeans.

TABLE 12.—*Calculated and observed coefficients of gas diffusion.*

Gas pair.	Calculated from above formula.	Observed.		
		Jeans.	Kaye and Laby.	Smithsonian tables.
Oxygen–air	0.180	0.178	0.178	0.178
			.171	.171
Oxygen–nitrogen	.177	.174	.677	.179
			.681	
Oxygen–hydrogen	.690	.679		.679
Oxygen–carbon dioxid	.131	....	.18	.136
				.180
Oxygen–carbon monoxid	.178	.183	....	.180
				.187
Carbon dioxid air	.131	.138	.142	.142
				.136
Carbon dioxid hydrogen	.517	.538	.55	.544
			.538	.538
Carbon dioxid carbon monoxid	.131	.136	.131	.131
			.141	.141
Carbon dioxid nitrous oxid	.095	.098	.1	.098
			.15	....
Hydrogen–air	.671	.661	.66	.634
				.661
Hydrogen–carbon monoxid	.661	.642	.649	.649
			.642	.642
Hydrogen–ethylene	.410	.486	.483	.486
Hydrogen–nitrous oxid	.510	.535	.535	.535
Carbon monoxid–ethylene	.117	.101	.101	.101

It is safe to conclude that the coeficient of diffusion of oxygen in helium is approximately 0.59. Since theory and experiment agree in fixing the coefficient of diffusion of oxygen in nitrogen at approximately 0.18, it follows that the diffusion of oxygen in helium will be, under like conditions, a little more than three times faster than in nitrogen. In a limited atmosphere, such as in the tins or culture tubes of these experiments, this means that a difference in oxygen percentage created locally, as, for instance, when oxygen is used up by the living root, will be equalized in helium in less than one-third the time which will be required in nitrogen. This effect is obviously in the direction of the biologic differences observed. It is the only intrinsic physical difference between nitrogen and helium known to us and which is in this direction. A test of quantitative correspondence between the actual rates of diffusion in the culture tubes and the observed differences in oxygen requirement, while theoretically possible, can not be made from the very approximate data now available for the oxygen requirement. This must await experimentation of greater precision.

If the hypothesis that the observed effects are due to diffusional differences be accepted, especial interest lies in the results on germination of Canada field pea and on stigmatic closure in *Diplacus*. It is easy to understand that diffusional differences may be regnant in the soil, where all gas diffusion is hindered by the soil particles. But in the germination tests the seeds were exposed freely to the atmospheres of the flasks, and with *Diplacus* the stigma was not only freely exposed but was surrounded by a rapidly moving atmosphere of the desired composition. In these cases an important difference of diffusion or gas composition in the inclosed atmosphere as a whole is unthinkable. If any other hypothesis as to the cause of the phenomena were available, these facts would constitute a serious objection to the diffusion hypothesis. Possibly they do anyway, but it is conceivable that the effective factor in these cases may be gas diffusion within the plant tissue or through the layer of gas immediately in contact with the living surface. There is little question that next to the actual solid, or liquid, surface of the plant there is a layer of gas held by adsorption and which may be many molecules thick. The composition of this layer will be determined by its dynamic equilibria with the plant tissue and with the surrounding atmosphere. It is reasonably certain that the composition of the adsorbed layer will be different when the surrounding atmosphere is helium and when it is nitrogen. It is not at all impossible that this blanket of adsorbed gas may play a larger rôle in the gas exchange of living matter than has been ascribed to it. While such a suggestion is purely speculative at present, it is a suggestion which appears to follow logically from the results presented herein.

In the soil, the rôle of diffusion rests on a less speculative basis. The conclusion from the present experiments is in harmony with present theories and with known data. Indeed, the hypothesis that the spontaneous diffusion of oxygen in the smaller passages of the soil is an essential element of soil aeration explains a cultural observation otherwise difficult to understand. It frequently happens that cultivated plants, especially potatoes and the legumes, show root injuries and nutritional disturbances suspiciously similar to injuries known to be due to deficient soil aeration. This is especially frequent on soils of close texture. In spite of this, the soil-air seldom, if ever, has a general oxygen-content below 10 per cent of oxygen by volume, and this concentration is much more than sufficient, under experimental conditions, for the roots of the plants in question.[1] This discrepancy, previously very puzzling to us, is explained by the importance of diffusion within the soil. In heavy soils it might easily happen that small portions of the soil become exhausted in oxygen to the point of

[1] See also p. 152, where the effect of temperature of the soil on the oxygen requirement of roots is discussed.

root injury, while other portions contain still a sufficient supply. Diffusional effects explain also another observation frequent in our previous experiments on soil aeration. When a root system begins to be injured by deficiency of soil oxygen, the initial injury is always apparent only in a few roots or portions of roots here and there throughout the root system. The remainder of the root system is apparently healthy. If the anaerobic conditions are continued, other roots and portions of roots succumb successively, until all show injury. If the experiment is stopped early in the progress of the injury, the injured roots die, but the uninjured ones do not do so unless the sum total of injury is sufficient to kill the entire plant.

In any biological consideration of gas diffusion, in soils or elsewhere, it must be remembered that the diffusion of carbon dioxid is to be reckoned with as well as that of oxygen. The same formula used above yields for the coefficients of diffusion of carbon dioxid in nitrogen and in helium 0.130 and 0.517 respectively. The difference in the rates of diffusion of this gas is even greater than in the case of oxygen. While accumulation of carbon dioxid because of deficient diffusion was probably not a significant factor in the present experiments, it might become so in other cases, especially in the soil.

A collateral conclusion from the data presented herein, as well as from previous experiments on soil aeration, is that plant roots apparently can use oxygen from atmospheres of great dilution. Instead of there being a certain percentage or partial pressure of oxygen below which the element is in some way unavailable to the root, the only important matter appears to be that oxygen be supplied at a sufficient rate. A very small percentage in the atmosphere is sufficient if the atmosphere is changed rapidly enough. Accordingly, any determination of "oxygen requirement" for roots, and probably for other tissues, is really a determination of minimum rate of supply, not of minimum percentage or partial pressure.

SUMMARY OF SECTION.

(1) Experiments with nitrogen containing approximately 0.5 per cent of oxygen and with helium containing approximately 0.2 per cent of oxygen showed differences in the effect of these gases on the life of roots of potato, the growth-rate and life of roots of Canada field pea, the germination of Canada field pea, the germination of rice, the growth of seedlings of sunflower, the respiration of unripe tomatoes and of germinating soy beans, the diurnal and other movements of the leaves of *Oxalis* and an acacia, the stigmatal movement of *Diplacus*, and several other plant activities. In all cases the nitrogen was less favorable to the vital activity than was the helium. With potato and Canada field pea this difference disappeared when the small percentages of oxygen contained in the gases were removed.

(2) No differences were encountered in the effects of the two gases on the life of the roots of *Coleus blumei*, the root-growth of maize, or the growth of seedlings of mustard.

(3) Experiments on the root-growth and germination of Canada field pea and on the movement of *Diplacus*, in which experiments use was made of nitrogen and of helium containing various known percentages of oxygen, showed that the percentages of oxygen required by these species for the vital activities studied were higher when the surrounding gas was nitrogen than when it was helium.

(4) These results are tentatively ascribed to the fact that the rate of diffusion of oxygen in nitrogen is less than one-third of its rate in helium, and it is suggested that variations in the diffusion of oxygen and of carbon dioxid may have importance in vital phenomena generally, and especially in the relation between root-growth and the aeration of the soil.

ROOT-GROWTH IN RELATION TO A DEFICIENCY OF OXYGEN OR AN EXCESS OF CARBON DIOXID.[1]

OUTLINE OF GENERAL EXPERIMENTS.

What constitutes "poor" aeration of the roots of land plants is a relative term and loosely characterizes the composition of the soil atmosphere as being on the one hand deficient in oxygen and on the other as having an excess of carbon dioxid. It connotes, in the first instance, a rate of oxygen delivery to the root which is not sufficient to meet the normal demands of the root. But the amount of oxygen required may be a specific matter, or it may vary with other features, as, for instance, the rate of growth itself, and possibly other physiological characteristics. From this it follows that whatever affects the growth-rate also modifies directly the oxygen requirement. Of these, temperature is of importance and its relation to the need of the root for oxygen will be taken up later in the section on the aeration relations of roots. What may constitute an excess of carbon dioxid may also be specific, but may also be associated with other factors external to the plant, as, for example, the partial pressure of oxygen in the soil atmosphere and the temperature of the soil. And again, what may be an excess of carbon dioxid at one stage in the development of the root may not be so at another stage. To make sweeping statements relative to soil aeration from a physiological point of view is, for such reasons, clearly not warranted.

The test of inadequate root aeration here used, and which is understood to apply only to the species referred to and under the conditions of the given experiments, is whether the rate of elongation of the root, and usually the main or tap root is meant by this, is less than that which would be expected to take place under conditions of normal soil aeration. In the course of the experiments no attempt has been made to calculate the variation of the root system in volume, but the relation of the root to the "grand period" of growth has been borne in mind. It will be shown, however, that, in the case of cotton at least, there is a very definite relation between the slowing of the growth-rate of the primary root and the appearance of secondary roots, and further, that for a limited time the mass of the latter approximately equals that of the former. Very shortly, however, this relation is changed and the volume of the laterals becomes continuously greater than that of the main root, and the curve of growth of the roots taken altogether does not exhibit the "grand period," but only a fairly continuous rise as plotted against time. It is probable, however, as suggested by Priestly and Evershed,[2] that

[1] By W. A. Cannon.

[2] Growth studies. I: Quantitative study of the growth of roots. Ann. Bot., 36: 239. 1922.

the rise is not strictly continuous, but is in part somewhat flattened as a result of the formation of other subsidiary rootlets. Thus S curves would result. Thus, what effect inadequate aeration would have on the growth of the entire root system was not investigated, although there does not seem to be a good reason for supposing it would not be similar to the effect on the main root itself, being different only in degree. This, however, should be studied directly for the reason, as is shown more than once in the course of the work, that the laterals may exhibit "acclimatization," or adjustment to differences in the oxygen-carbon dioxid content of the soil, not possible for the main root itself. This may be particularly true when the life of the latter is more limited than that of the former, either naturally or only during and owing to the nature of the experiment. It, indeed, is thought that such differentiation in reaction may, in some instances, underlie marked development of laterals as opposed to the limited development of the main root in certain species growing under natural conditions.

The general procedure has already been described in sufficient detail and need not be mentioned further in this place than to state that where any noteworthy deviation from the customary method was made, as, for instance, in the employment of the horizontal microscope to watch the immediate effect of a gaseous mixture, such will be characterized, or at least alluded to. Finally, in the running account it is the aim to give, not a transcription of a laboratory note-book, but sufficient detail to indicate in what way the results were derived, that is to say, the general course of the experiment.

Allium cepa.

In the experiments with the garden onion, small sets were used which were either started in the tubes or were transferred to the tubes after having sent out roots. The experiments were carried on in the cellar of the greenhouse laboratory at Carmel and in the greenhouse above. In the former the temperature of the air during the experiments remained about 20° C., but in the greenhouse the daily range was considerable, being both above and below 20° C. In any event, however, the temperature of the roots was kept approximately constant.

Owing to inconsistent results, to be characterized below, it was found advisable to use especial methods, particularly as regards the placing of the seal. At the beginning all of the parts, which normally occur below ground, were included within the seal; that is, the seal was placed immediately above the bulb. In this, as will be seen, the bulb is included with the roots and lies below the seal. The early results lead to the suspicion that there was an inward leakage of oxygen when the seal was placed in this manner. But it should be remarked that the seal itself was quite tight under any circumstances, and that the gaseous mixtures employed, which were under slight pressure, were static when it was desirable to have them so. It appeared, therefore, that there was possible inward diffusion through the scales of the bulbs if not inward molar flow between them. Finally, the bulbs were well coated with vaseline or grafting-wax, and the

plasticine seal was placed below the bulb, that is, at the root crown, which stopped the inward movement of oxygen. An analogous oxygen leakage, it should be remarked, was observed in *Potentilla anserina*, as will be mentioned elsewhere.

In a preliminary experiment, the rate of root-growth was observed in normal soil atmosphere, when this was static, and also when it was caused to move gently through the soil of the culture. In one such test, in which the root was under observation for 48 hours, static atmosphere was used during the first half and moving atmosphere during the second half of the period. The total growth was 8 mm., of which 3 mm. took place in static air and 5 mm. in the gently flowing air.

The next experiment with onion was with a soil atmosphere containing 2 per cent oxygen, with the balance nitrogen, which was alternated with normal atmospheric air. While in the experimental atmosphere the root increased 2 mm. in 24 hours. But in this instance, as is frequently to be noted in roots growing in a deficient oxygen-supply, the root turned sharply from the vertical and took a course nearly at right angles to that previously followed. Following this treatment, atmospheric air was used, static, for 24 hours, with the temperature conditions the same as before. During this period the root increased 6 mm. in length; after this, 2 per cent oxygen was again used, and continually so for 72 hours. No growth of the root occurred during this time. Finally, air was again admitted and the root was kept under observation for 48 hours under such normal conditions. As a result, it was seen that during the first 24 hours in normal soil atmosphere no additional growth took place, but during the last 24-hour period the root increased 8 mm. in length.

In a second introductory experiment the root of No. 1 was seen to grow 3.5 and 4 mm. each day during 48 hours while in normal soil atmosphere. A gaseous mixture consisting of 2 per cent oxygen with nitrogen was then administered for 6 consecutive days. Daily growth in this experimental atmosphere was noted as follows: 2, 4, 1.5, 2, 0, and 0 mm. At the expiration of the first 24 hours the root was seen to have turned at an acute angle, as in the instance above given, and was directed sharply away from the vertical. It was assumed that in each instance the root had lost its capacity for geotropic response, owing to the deficient supply of oxygen. The culture was then given atmospheric air for 24 hours, but growth was not renewed.

Plants Nos. 2 and 3 were subjected to the same treatment as No. 1 and the behavior of the roots was observed at the same time. In normal soil atmosphere the roots of these plants increased 2 mm. and nearly 5 mm., respectively, in length in 24 hours. The cultures were thereupon given 2 per cent oxygen continuously with the following results: In No. 2, which had two roots in view, 1 and 5 mm. growth took place during the first 24 hours and none subsequently. In No. 3 a growth of 4 mm. took place the first 24-hour period, 2 mm. the second 24-hour period, and none thereafter. Following the treatment with deficient oxygen-supply, both cultures were aerated during 24 hours. Resulting from this it was found that growth occurred in one root of culture No. 2, which increased 2 mm. in length, but none in No. 3. Finally, culture No. 2 was given 1.5 per cent oxygen when growth of the roots ceased.

In the second series of experiments plants Nos. 5, 6, 7, and 8 were used. In this series the rate of growth was observed with the roots in normal soil atmosphere, in 4 per cent oxygen, in 2.4 per cent oxygen, and in 1.8 per cent oxygen, with the balance nitrogen in each instance. Finally, normal atmospheric air was again used.

At the beginning of the series, and before the experimental atmospheres were employed, a daily root-growth from 2.5 to 10.5 mm. was seen to take place, although growth doubtfully occurred in No. 6. At the end of the preliminary observations with the roots in normal soil atmosphere, 4 per cent oxygen was administered for 72 consecutive hours. During this period a daily growth between 2.5 and 5 or 6 mm. occurred. Moreover, it was noted that the amount of growth on the last day of the test was quite as much as on the first day.

Immediately following the use of 4 per cent oxygen, a gaseous mixture containing 2.4 per cent oxygen was given for 48 hours continuously. It was observed that during the first 24-hour period the amount of growth was 2.5 to 3 mm., but that for the second day it was 1.5 to 2 mm., or that it had fallen off slightly.

At the close of the last experiment 1.8 per cent oxygen was given for 48 hours as before. As a result, it was observed that plants Nos. 5 to 8 had for the two days the following root-growth: 0.5, 1.5, 1, and 2 mm. the first day, and 0, 0.5, 0, and 1 mm. the second day, respectively.

Finally the cultures were aerated and the root-growth for 24 hours was noted while the roots were in normal soil air. As a result, a certain amount of growth was seen to occur in each root, the amount for each of the four plants being 2.5, 1, 1, and 3 mm.

In a third series of experiments with the onion, plants numbered 9, 10, 11, and 12 were used. In this series root-growth in normal soil atmosphere was demonstrated, after which gaseous mixtures containing 1.8 per cent oxygen and 1.5 per cent oxygen, with the balance nitrogen, were given, followed finally by atmospheric air.

While in normal soil atmosphere for 24 hours, at the beginning of the series, a root-growth from 2.5 to 8 mm. was observed, although in No.10 growth was in doubt.

The experimental atmospheres with deficient oxygen were given 5 days, of which the gaseous mixture with 1.8 per cent oxygen was used the first 4 days. In this atmosphere no or very slight growth was demonstrated for cultures Nos. 9 and 10, but in the other two a daily growth of 1.5 to 6.5 mm., for No. 11 and of from 2 to 3 mm. for No. 12 took place. On the fifth day 1.5 per cent oxygen was given and the cultures were left in this mixture 24 hours. In the atmosphere last named no root-growth took place in Nos. 9 and 10, but the root of culture No. 11 increased 4.5 mm. and of No. 12, 2.5 mm. in length.

Finally, the cultures were aerated and the growth of the roots was observed for 24 hours in normal soil-atmosphere. During this time the roots of Nos. 9 and 10 showed no signs of recovery, but those of Nos. 11 and 12 increased 4.5 and 2.5 mm. in length.

The unexpected behavior of the roots of Nos. 11 and 12 pointed to the possibility that these cultures had in some manner received more oxygen than the other two. This would occur if the gaseous mixture streamed slowly through, or if there was a leak in the seal of *both* culture tubes. But in the former case gas passage would be detected by a wash-bottle for that purpose, and in the latter gas leak would be noticeable by its loss in the gasometer. In any event, what happened to one culture would have no effect on the rest, as each culture had separate connection with the distributer. What made leakage, or other means whereby the oxygen-content of the culture tubes was increased, the more probable was the further fact that upon being aerated there was no apparent increase in the rate of growth of the roots. Thinking that possibly there may have been downward

passing of atmospheric air by diffusion through the shoots of two of the plants and not of the other two, the leaves of Nos. 9 and 10 were cut across, the better, possibly, to admit air to the interior, and thus to give it possibly quicker or better access to the roots. This, however, had no apparent effect on the roots.

In the experiments referred to, the bulb was placed below the level of the soil, and the seal of plasticine was therefore above it. Under such circumstances there was no outward gas leak, but the possibility remained that there might be inward diffusion through or between the scales of the bulb, whereby the roots might receive a larger supply of oxygen than that provided by the experimental atmosphere. That such, in fact, is probably what occurred is indicated by the following, which is representative of several experiments. The bulb was coated with grafting-wax and afterward packed with plasticine, so that outward leakage was not possible. On May 31 the culture was given 1.2 per cent oxygen for 24 hours. On June 1, five successive gas samples, of 50 c. c. each, excluding the first, taken from the tube, gave the following percentages of oxygen, namely, 1.8, 1.6, 1.4, and 1.8. After more gas had been taken from the culture, the amount of oxygen was found to be 1.2 per cent That this was owing to inward leakage, or diffusion in connection with the plant, was probable from the fact that successive oxygen-determinations with blank tubes made at another time, but under quite similar conditions, did not vary more than 0.1 per cent after the second 50-c.c. sample, which was 0.2 per cent low, had been taken.

In certain experiments also, as would be expected, where the amount of the root mass was relatively large, oxygen analyses showed a slightly less amount of that gas present in the tube than was in the gas-tank. Thus, on May 10, a gaseous mixture containing 3 per cent oxygen was given for 24 hours. At the end of that period successive analyses of 50 c. c. each, except the first 50 c. c., gave 2.2, 2.8, and 3.0 per cent oxygen. The inward diffusion was clearly insufficient to make up for the amount absorbed by the relatively extensive root system and was masked by this fact.

The inconsistencies in results above referred to had to do with irregularities in the oxygen-content of the soil whereby the roots of some cultures had a better oxygen-supply than those of others growing under apparently identical conditions respecting that gas. But an additional irregularity, not explainable by this, was also noticed. Thus, onion cultures served with the same gaseous mixture, but growing under unlike soil temperatures, might react differently and in a manner not to be accounted for wholly by the temperature differences. For example, at a soil temperature of 21° C., root No. 2 of a culture grew 10 mm. in 24 hours, while at the same time but at a soil temperature of 16° C., root No. 2 of another culture increased 5.5 mm. in length. Both cultures were then given a gaseous mixture containing 1.6 per cent oxygen for 24 hours, when it was found that the growth of the root first mentioned had fallen to 2.5 mm. and that of the second culture to 3 mm. The growth index of former,

$$\frac{r}{R} \cdot \frac{\text{growth in the experimental atmosphere}}{\text{growth in normal soil atmosphere}}$$

of the two roots was therefore 0.25 and 0.54. This indicated that the oxygen requirement of the roots of the onion is less at a soil temperature of 16° than at a soil temperature of 21° C. In an additional experiment in which the percentage of oxygen was 2.8 to 3, the r/R at a soil temperature of 27° C. was found to be 0.80 to 1. It seems probable, therefore, that in a gaseous

mixture containing a given oxygen deficiency, less than 3 per cent of that gas, the growth index of onion root varies inversely as the temperature, between 16° and 27° C. Further, it is probable that at a constant temperature, the growth index, as would be expected, varies directly with the amount of oxygen in the soil, at least between 1.6 and 3 per cent.

Avena sativa.

A single series of experiments, in which four seedlings were used at the same time and subjected to similar treatment, was run with a species of the cultivated oat. In the experiments cylinder nitrogen only was used. In this, as elsewhere shown, there is about 0.5 per cent oxygen.

The experiments ran for more than 2 weeks continuously, during which time the gaseous mixture was employed statically and placed under slight pressure. During the first week the temperature of the cultures was not under control, but varied during each day, and from day to day, after that of the greenhouse, but during the second week the cultures were kept at about 21° C.

It should be especially mentioned that there was usually more than one root in each culture which was under observation, so that the variation in reaction to the diminished supply of oxygen, which will be apparent from the running account, may have to do not only with different plants, but with different roots of one and the same plant.

The reaction of the roots was various. In culture No. 1 a marked root increased 4 mm. in length during 24 hours just preceding the administration of the experimental mixture. The culture was then given 0.5 per cent oxygen and the growth fell to 1 mm. during the first day in the mixture, when growth ceased.

In culture No. 4 a root-growth of 12 mm. occurred during 24 hours while still in normal soil atmosphere. During the first 24-hour period in the experimental mixture the growth was 2 mm. Thereafter for 5 days no root-growth occurred, but from the sixth to the eleventh day slow growth again took place. After the eleventh day no further root-growth was recorded.

In cultures 2 and 3 there was a certain amount of root-growth during the first 10 days, after which growth ceased. This, however, was not true of every root of each culture, but of some root in each culture. In No. 1, for example, root marked No. 1 grew daily during the first six days, when growth ceased. Root No. 2 of the same culture, however, increased in length for 10 consecutive days before growth stopped. On the fifth, seventh, and eighth days the growth amounted to 8 mm. each 24 hours. The behavior of culture No. 3 was essentially the same as in the culture last described.

In general, it appears that the roots of the variety of oat used are relatively insensible to oxygen deficiency, although there seems to be individual variation in this regard, not accounted for by the experiments, as well as a marked variation as between roots of the same individual.

Baccharis pilularis.

Baccharis pilularis is a highland species which occurs in the neighborhood of Carmel, as distinguished from *B. viminea*, which grows along streamways and in other moist situations. The soil in which the species occurs, therefore, is a well-aerated one and relatively warm.

On October 31, an experiment was set up in which *B. pilularis* and *viminea*, *Salix lasiolepis*, *Zea mays*, and *Alnus rubra?* were given parallel conditions of temperature and of soil aeration. The temperature of the soil

was for the most part 24° C., and 1.6 per cent or less oxygen was used. In recounting the history of the experiment only casual reference will be made to species other than *B. pilularis*.

In normal soil-air during 24 hours just previous to the gas administration, the root of *B. pilularis*, at a temperature of 24° C., increased 18.5 mm. in length, and at the same time the root of *B. viminea* grew 3.5 and that of *Salix* 10 mm. Then 1.6 per cent oxygen was given the cultures 3 days continuously, with the result that the growth of *B. pilularis* was seen to be 2.5, 3, and 3 mm., and that of *Salix* was 3.5, 3.5, and 3 mm. for the same daily periods. On the last day referred to, *Alnus* had a root-growth of 3.5 and 5 mm. while in normal soil-air and at a temperature the same as the other cultures. The root of *B. viminea* turned from the wall of the glass culture tube and was not seen for about 14 days, during which time, however, the culture was treated quite as the rest.

All of the cultures were opened November 4 and irrigated with tap-water, after which the gas was renewed with the same oxygen partial pressure as before. This was continued 2 days. During this time the root of *B. pilularis* grew 0.5 mm. each day, that of *Salix* grew 5 and 4 mm., and of *Alnus* 1 and 0 mm.

The cultures were irrigated November 6 and left in normal soil-air for 24 hours, at which time the root-growth in *Zea* was observed in addition to that of the species above mentioned. During this period the roots of *B. pilularis* and of *Salix* each grew 6 mm. and that of *Alnus* 2.5 mm.

On October 7 the gas administration, again with 1.6 per cent oxygen, was repeated. This was continued for 6 days with various results. For 72 hours the daily root-growth in *B. pilularis* was 5.5, 8.5, and 5 mm. On the second day an analysis showed 0.4 per cent oxygen in this culture. During the following 2 days the root of the species grew more than 1 mm. each day. On the last day the tip was hidden from view.

At the end of the last period of gas administration, the cultures were aerated for 24 hours and then were given the same gaseous mixture as before, but it was slowly streaming, at the rate of 1 c. c. per minute, through the culture of *B. pilularis*. Owing, however, to the fact that the root-tip was hidden, as above remarked, the immediate effect on root-growth could not be directly measured. During this stage of the experiment a daily root-growth of 2.5 and 3 mm. took place in *Alnus* and in *Salix*.

The experiment was reset and all of the cultures were aerated for 24 hours. Two cultures of *Zea*, not previously used, were added to the experiment. Of these, No. 1 had a root 12.5 cm. and No. 2 a root 21 cm. long. A cutting of *Salix*, which also was fresh, replaced the one previously used in the experiment. The temperature of the soil was 24° C. The amount of growth of the roots of the cultures, while in normal soil air for 24 hours before the gas administration, was as follows: *B. pilularis*, 6.5 mm.; *Zea*, No. 1, 26 mm., and No. 2, 35 mm.

The cultures were then given the 1.6 per cent oxygen, streaming, at the rate of about 1 c. c. per minute, for 72 hours. As a result, it was observed that root-growth in *B. pilularis* was 1.5, 3, and 1 mm. for each of the 3 days. In *Salix*, 1.5 mm. growth took place on each day. In *Zea* No. 1 the root-growth for the first 48 hours was 11 mm., but only 1.5 for the final 24-hour period. In the second *Zea* culture a daily growth of about 2 mm. occurred.

The experiment was again reset and the cultures irrigated and aerated. At this time, and for 24 hours in normal soil air, the root of *B. pilularis* increased 3.5 mm. in length; that of *Salix*, 5.5 mm.; and that of *Zea* No. 1,

24.5 mm. and No. 2, 32.5 mm. All of the cultures were then given 0.8 per cent oxygen, with nitrogen, for 4 days continuously. The gas was strictly static. Root-growth of the cultures was as follows: *B. pilularis*, 3.5, 2.5, 2.5, and 2 mm.; *Salix*, 5.5, 5, 6, and 5 mm.; and in *Zea* one of the cultures was not tight the first day when 7 mm. growth occurred, and the other was not tight any day and the root grew daily from 5.5 to 18.5 mm.

The experiment was reset again and all of the cultures irrigated and aerated. The root-growth in normal soil-air for 24 hours was found to be 1.5 mm. in *B. pilularis*, 8.5 mm. in *Salix*, and 12 and 10 mm. in *Zea* Nos. 1 and 2, respectively. The cultures were then given 1 per cent oxygen for 24 hours, followed by 0.5 per cent oxygen. No further root-growth was noted in *B. pilularis*.

Thus the series of experiments show that the oxygen requirement of *B. pilularis* is not far different from that of *B. viminea* or *Salix lasiolepis*, and is greatly below the requirement of *Zea*. Growth of the root can go on in 1.6 per cent oxygen, but stops when the partial pressure of oxygen falls to 0.5 per cent. The fact that the roots of *B. pilularis* are able to maintain growth in a low percentage of oxygen is of interest because, as pointed out above, the species naturally occurs in well-aerated soil, from which it might be concluded, but wrongfully as it appears, that the roots would be intolerant of poor soil aeration.

Baccharis viminea.

Baccharis viminea occurs on the lowest bench by the Carmel River, along the banks of the river channel, and, nearer Carmel, in similar well-watered situations. In preparing the species for study, cuttings were made and left in water until roots began to show, when they were transferred to culture tubes and handled thereafter in the usual way. In all of the cultures the shoot was well covered with leaves. The soil temperature of the cultures was kept between 19° and 23° C.

In the studies on the aeration relations of *viminea* especial attention was given the possibility that, growing in so moist a situation, the roots of the species might be expected to endure successfully a soil poor in oxygen or rich in carbon-dioxid. In attempting oxygen-free experiments it was found extremely difficult in practice to absolutely exclude that gas. Passing cylinder nitrogen slowly through three wash-bottles containing alkaline pyrogallol did not remove all of the oxygen, which was of small amount at first, and finally, it was found necessary to make use of an electric reducing-oven, with other precautions not necessary to present in further detail.

At the beginning of the experimentation with *Baccharis*, the rate of growth was learned with the roots in normal soil atmosphere. In a 24-hour period this was as follows: culture No. 1, in which two roots were visible, 7 and 8 mm.; in No. 2, 7 mm.; in No. 3, with two roots in view, 7 and 5.5 mm.; and No. 4, also with two roots to be seen, 9.5 and 5.5 mm.[1]

Immediately following the use of atmospheric air, a gaseous mixture consisting of 0.5 per cent oxygen, balance nitrogen, was given all of the cultures for a period of 22 days. It was found that, with little exception, some growth occurred every day in all of the roots measured. There was, however, considerable variation in the amount for 24-hour periods. In culture No. 1 the daily growth-range was between 2 and 9 mm., with an average of about 3.5 mm. each day, for the entire 22 days. In No. 2 the greatest difference in the daily rate, but possibly not of the same root, was

[1] The numbers of the cultures refer only to the experiment being described and not to the entire series on *Baccharis*.

1 and 13 mm. In No. 3 the growth range was about the same as in No. 2. In No. 4, however, the range of growth for 24-hour periods was the most of all, the extremes being 2 and 18 mm., with a daily average of 6 mm. A comparison of the root-growth of the first and the twentieth days in the experimental soil atmosphere is instructive. At the end of the first 24 hours the following increases in root-length were noted, namely, in No. 1, two roots, 4.5 and 9 mm.; in No. 2, two roots, 9 and 3 mm.; in No. 3, 10 mm.; and No. 4, two roots, 5 and 18 mm. The root-growth for the 24 hours ending the twentieth day, for cultures 1 to 4, was, respectively, 5, 0, 4.5 and 5, 3, and 3 mm. In No. 2 growth occurred on the twenty-first day, and not the twenty-second, and in No. 1 it was not seen after the twentieth day. In both No. 3 and No. 4 growth was observed the twenty-second day.

The first series of experiments on *Baccharis* appears, therefore, to show that the roots can maintain a fairly active growth for at least 22 days in a soil which does not contain over 0.5 per cent oxygen. That no serious injury was done to the roots appeared from the behavior of cultures Nos. 1 and 2 when given atmospheric air for 5 days immediately following the treatment last described. One root of No. 1, for example, increased in length 26.5 mm. and a root of No. 2, 25 mm. during this period.

In the second series, cultures Nos. 3 and 4 were given cylinder nitrogen after it had passed slowly through three wash-bottles of alkaline pyrogallol. The experiment lasted 7 days. The following daily increments in root-length, while in the gas, was as follows: In No. 3, which had two roots in view, the growth of one root for the period was 6.5, 4, 3, 3.5, 4, 2.5, and 0 mm., and that of the other was 5, 4, 4.5, 5, 4.5, and 5.5 mm. The growth in No. 4, in which two roots also were measured, was for one root 5, 6, 7, 5, 5, and 6.5 mm., and for the second root 3, 5, 4.5, 3.5, 6.5, and 6.5 mm. It was evident, therefore, either that the roots of the plant were capable of growth under anaerobic conditions, or that there was oxygen present, however small the amount might be.

In order to surely exclude all oxygen, an additional series of experiments was started in which cylinder nitrogen was passed through an electric reducing furnace, and in which many precautions were taken to exclude the possibility of an inward infusion of the gas into the culture tube itself.

In this, the third series, the cultures were divided so that in the first experiment Nos. 1 and 2 were given atmospheric air, while at the same time and under the same conditions oxygen-free nitrogen was administered to Nos. 3 and 4. In the second experiment the conditions were reversed; that is, Nos. 1 and 2 were kept under anaerobic conditions while Nos. 3 and 4 were aerated.

Cultures 3 and 4, in the first part of the experiment, were given oxygen-free nitrogen for 5 consecutive days. The effect on the growth of the roots was marked and positive. In each culture two roots were in view. An examination of the cultures at the end of the first 24 hours showed that the roots of No. 3 had grown 3 and 3.5 mm., and that those of No. 4 had grown 2.5 and 3 mm. Subsequent to this, however, and during the remainder of the experiment no root-growth took place in either culture.

Immediately after ending the experiment with oxygen-free nitrogen, the cultures were aerated and left in normal soil atmosphere for 6 days. In both cultures root-growth occurred daily, although it was least during the 24 hours first following the use of the nitrogen. One root of culture No. 3, during the 6 days, increased 24 mm. in length and those of No. 4 grew at about the same rate.

In the second experiment of the third series, the effect of anaerobic conditions on the root-growth of cultures Nos. 1 and 2 was observed. At the beginning of the experiment the roots of the cultures were well aerated and the rate of growth noted. In No. 1, with two roots in view, one increased 28 mm. in length in 5 days, and the other root grew somewhat less. Growth of the roots of No. 2 was also demonstrated while in normal soil air.

After having been under observation for 5 days, both cultures were given oxygen-free nitrogen, as in the first experiment. This was continued for 7 consecutive days. As a result, it was seen that no growth occurred during the first 3 days, 1 mm. was doubtfully recorded for the fourth day, and none thereafter.

It seems quite clear, therefore, that the roots of this species of *Baccharis* can maintain a slow rate of growth in as little as 0.5 per cent oxygen, but that upon its total removal growth ceases.

A fourth series on the aeration relations of the roots of *Baccharis viminea* was carried out in which the direct effect of an excess of carbon dioxid in the atmosphere of the soil was observed. In these experiments cylinder carbon dioxid was used, either diluted with atmospheric air or cylinder nitrogen, or it was used undiluted. In any event, the amount of oxygen present was sufficient to permit growth of the roots where the diluting gas is innocuous.

Previous to the gas administration and while in normal soil atmosphere, root-growth in cultures 5, 6, and 7 was observed for 6 days. In each of the cultures two roots were in view. In No. 5 the total growth was 7 mm., in No. 6 it was 12 mm. for each root, and in No. 7 it was 20 and 26 mm. Thus the average growth for 24-hour periods was for the cultures, respectively, 1.2, 2, and 4 mm.

In the first test, 25 per cent carbon dioxid, with the balance atmospheric air, was employed. This was continued 2 days. As a result it was noted that in No. 5 one root increased 3 mm. and the second 8 mm. in length, in No. 6 the roots grew 4 and 8 mm., and in No. 7 the growth was 8 and 9 mm. Thus the daily average was in fact quite as much as in normal soil atmosphere.

In a second series the amount of carbon-dioxid was increased to 50 per cent, which was given for 72 hours. Two roots in each culture were measuured, with the following results: In No. 5 one root showed a growth of 4.5 mm. the first day, 2.5 mm. the second day, and none the third day of the experiment. The second root of the same culture increased in length each day as follows: the first day 2 mm., the second day 1.5 mm., and the third day 3.5 mm. In No. 6 root-growth occurred during the first 48 hours only. In the last instance that of the second and of the third day was little.

At the close of the last experiment the cultures were aerated and were left in normal soil atmosphere for 5 days, during which growth was seen to occur each day.

In a third series the same plants were used as in the preceding experiment, but the amount of carbon-dioxid was reduced to 10 per cent, with the balance cylinder nitrogen. In this experiment, therefore, not only was there a relatively large amount of carbon dioxid, but there also was a small amount of oxygen, as distinguished from the earlier experiments in which the former was used with atmospheric air, and hence in which the oxygen partial pressure was fairly high. The experiment lasted 96 hours. It was seen that root-growth continued each day, although the amount averaged only about 1 mm. for each 24 hours.

To complete the series of experiments on root-growth in an excess of

carbon dioxid, a final experiment was planned in which cylinder carbon dioxid was employed. In this, the fourth experiment, cultures 1 and 2 and a culture not previously studied were used.

Cultures 1 and 2 were employed in the first part of the fourth series. The root-growth of these plants, two roots in each, during 24 hours immediately preceding the gas administration, was as follows: No. 1, 2 and 10 mm., and No. 2, 5 and 6 mm. Both cultures were given the gas for 48 hours, with the following effect on the rate of root-growth: In No. 1 culture one root did not grow during the first 24 hours and the other root increased 0.5 mm. in length. In the other plant, however, 2 and 3.5 mm. growth took place during this time. In the last 24 hours the growth of No. 1 was 1 and 0 mm., and that of No. 2 was 0 and 2 mm. It was observed that very shortly after the carbon dioxid was given, the shoots of both cultures became wilted. Before the end of the first day, however, both had recovered.

In the second part of the same experiment a vigorous cutting, not previously used, was given cylinder carbon dioxid for 48 hours. Growth of the roots of this plant ceased immediately upon the gas administration and was not renewed during the balance of the experiment.

The results of the experiments on *Baccharis viminea* indicate that the species is well adapted to successfully withstand conditions of exceedingly poor soil aeration. Thus, root-growth was seen to go on for a period of 3 weeks in a soil atmosphere which contained only about 0.5 per cent oxygen, although when entirely deprived of that gas, growth promptly stopped. The roots also are able to continue growing for a very considerable period of time in high percentages of carbon dioxid, although whether they can do so when oxygen is quite wanting was not determined and is doubtful. The rate of growth, however, in an absence of carbon dioxid is less than in normal soil atmosphere. It is probable that there are specific effects of the gas, and, also, it appears that to a certain extent immunity can be built up through the gradual increase in the amount employed.

Citrus spp.

Seedlings of the Brazilian sour orange, of the Valencia and Ruby sweet oranges, and of the rough lemon, supplied by the California Citrus Experiment Station, Riverside, were used in several experiments on the reaction of roots to differences in soil aeration. The gaseous mixtures, in addition to atmospheric air, included different proportions of oxygen-nitrogen, oxygen-nitrogen-carbon dioxid, and oxygen-carbon dioxid, and also oxygen-free nitrogen. The temperature of the soil ranged between 19° and 30° C. In a portion of the experiments, where a constant-temperature chamber was used, the root and the shoot were of the same temperature, but for the most part the temperature of the soil only was controlled.

Sweet Orange.

In the introductory series of experiments on the sweet orange, with a deficiency of oxygen, the amount of oxygen was between 0.5 and 1.4 per cent with nitrogen, and in tests with an excess of carbon dioxid, 2.7 to 21.8 per cent oxygen with nitrogen was used.

The first series was run in the constant-temperature chamber, where the temperatures were between 20° and 25° C. The object at first was to roughly determine the least concentration of oxygen at which root-growth would take place. Immediately preceding the first experiment the growth of the roots during 24 hours, in normal soil-air, was seen to be from 6 to 9 mm. at

the temperature used. Then 1.4 per cent oxygen, with 2.7 per cent carbon dioxid, and with the balance in nitrogen, was given the cultures 72 hours continuously. At the end of the period it was seen that the root of one specimen had grown 2 mm. and that of another 3.5 mm. Immediately at the close of the experiment the seedlings were given a mixture consisting of 0.8 per cent oxygen, 5.8 per cent carbon dioxid, and nitrogen, for 5 days. It was found at the end of this period that the root of No. 1 had grown 6 mm., while that of No. 2 had turned from the glass wall and the tip was lost to view. At the termination of the last test the same plants were given 1.3 per cent oxygen, 21.8 per cent carbon dioxid, and nitrogen during 48 hours. As a result it was found that the root of No. 1 had grown 4 mm. in the mixture.

The preliminary tests, therefore, appeared to indicate that the roots of the sweet orange are relatively insensitive to poor soil aeration, that is, to a great deficiency of oxygen and a relatively large amount of carbon dioxid. These results were pressed somewhat further in additional tests.

In the second of the introductory series the immediate object was to learn whether growth of roots of the sweet orange would continue in the presence of a large excess of carbon dioxid. As carried out, the actual amount of oxygen present was 3 per cent of the amount in cylinder carbon dioxid, or more than 3 per cent.

In the first experiment of the series, the mixture consisted of 25 per cent oxygen and 75 per cent carbon dioxid. It was administered 96 hours continuously. Growth of the roots went on throughout the period, but slowly. The entire growth was 6 mm.

Following the last test, cylinder carbon dioxid was given for 24 hours. The gas was static. A slight amount of growth, about 0.5 mm. occurred during the period. In another experiment, which was continued 72 hours, the growth of the roots was found to be about 2.5 mm. That, as a matter of fact, the carbon dioxid was in great excess, was shown by its power to extinguish a lighted match after having passed through the cultures.

The third of the introductory series was carried out without carbon dioxid, but with nitrogen, with or without oxygen. The gases consisted of 0.5 per cent oxygen with nitrogen, and oxygen-free nitrogen.

In the first of the tests, 0.5 per cent oxygen was used. During 24 hours just preceding the experiment the roots of the seedlings used increased 6 to 9 mm. in length. Then the gas was given for 5 days continuously, at the end of which period it was found that each of the roots of two cultures had grown 10 mm. At the same time and at the same temperature the root of the control grew 20 mm. Oxygen determinations were not made of the cultures in these tests.

The second experiment was run with nitrogen only. The oxygen was removed by passing the cylinder nitrogen through an electric reducing furnace, and then through three wash-bottles with alkaline pyrogallol. In the setting up of the test, the use of rubber tubing was avoided as much as possible, and other precautions were taken to insure the complete absence of oxygen, not only at the beginning but also during the course of the experiment. Before the oxygen-free gas was used, the normal soil atmosphere of the culture was swept out with cylinder nitrogen, after which the nitrogen, equal to several times the volume of the tubes, was run through. The tube was sealed and the gas placed under slight pressure and left for 24 hours. As a result it was found that although root-growth had been demonstrated immediately before the beginning of the test, no growth occurred in the strictly anaerobic conditions of the experiment.

It would appear from the introductory experiments that root-growth in the sweet orange is inhibited in the total lack of oxygen, but that it can go on, although slowly, in an excessive amount of carbon dioxid. Although these tests do not establish the possible minimum concentration of oxygen consistent with root-growth, and at any temperature, they seemed to indicate that it might be low. In additional experiments an attempt was made to learn at what soil temperature and in what partial pressure of oxygen growth of the roots would continue. These were carried out on the sweet orange as well as on the Brazilian sour orange and the rough lemon.

On April 16, three seedlings of sweet orange were prepared for study and were placed in the thermostat running at 21°. In 24 hours the root-growth of these was 1, 2, and 2 mm. Thinking the growth-rate low for the immediate purpose of the experiment, they were thereupon placed in thermostats at 30° and 27.5° C., where the growth in 24 hours became 3.5, 3.5, and 4 mm. for the same plants. They were then given 1.2 per cent oxygen for 24 hours. During this time the root of No. 1, Ruby orange, did not grow, neither did that of No. 2, Valencia, but the root of No. 3 grew 2 mm. Oxygen analyses showed that there was 1.2 per cent in the Ruby culture, 0.8 per cent in Valencia, No. 2, and 1.6 per cent in No. 3. The second and third lots of the gas sample in the last two cultures gave 1.2 per cent oxygen, showing that there had been a slight inward diffusion from the atmosphere. The same seedlings were then placed at a temperature of 18° and 20° C. and were given the same mixture, but there was no growth of the roots.

On May 30, a fresh lot of seedlings were used. Of these 2 were placed in the thermostat running at 20° and the growth of the roots in normal soil-air for 24 hours was seen to be 3.5 mm. in each. No. 1 was a Ruby and No. 2 a Valencia orange. The seedlings were then given 1.6 per cent oxygen, with nitrogen as before, for 24 hours, with negative results in both cultures. At the end of the period, analyses showed that the amount of oxygen in the tubes was from 0.2 to 0.6 per cent below the amount in the container. At the end of the test the cultures were aerated for 48 hours, but left in the same thermostats. There was no growth during the first 24 hours in normal soil-air, but 2 and 2.5 mm. the second day.

The results, thus, with the sweet orange are not consistent, and no explanation of the apparent difference in behavior is offered, except to point to the possibility of inward diffusion in the earlier experiment, which was not suspected and was not detected at the time. Assuming that such criticism can be made of the introductory work, it can be concluded that so far as the Ruby and Valencia oranges are concerned, and under the conditions of the experiment, the minimum concentration of oxygen for root-growth is probably high. Thus, it is above 1.4 per cent even at a soil temerature of 20° C.

Brazilian Sour Orange.

A few experiments were carried out on seedlings of the Brazilian sour orange, which, although not conclusive, appear to yield yet other results than those above remarked with species of the sweet orange. On August 20, six cultures were placed in three thermostats running at 27°, 22°, and 19° C., two in each. During 24 hours in normal soil-air the root-growth was as follows: 2.5, 2.5, 3, 7, 0.5, and 2 mm. for the six. Owing to the behavior of Nos. 4 and 5, they are not included in the account, leaving thus two cultures at 27°, one at 22°, and one at 19° C. Then 1.6 per cent oxygen was given the cultures for 24 hours. At the end of this time root-growth was found to be as follows: 2.5, 1.5, 1.5, and 1 mm. for the same

plants, with the omissions noted. The oxygen determination of the tubes at the end of the test gave the following for the respective cultures: 1.2, 0.8, 0.6, and 1.6 per cent. On the third day of the test the cultures were irrigated, and after having been 24 hours in normal soil-air the growth was found to have been as follows and for the same cultures: 0.5, 1, 1, and 0.5 mm. These results appear to indicate, therefore, that the minimum oxygen requirement for the sour orange is not so high as in the sweet orange. To learn whether growth in this species would go on in less than 1 per cent, other specimens were given 0.6 per cent oxygen. Two temperatures, 18° and 30°, were used. At the latter temperature, a growth of 7.5 and at the former a growth of 2.5 mm. was noted for 24 hours in normal soil-air. At the end of 24 hours in the gas with 0.6 per cent oxygen, however, it was found that no growth had taken place. Moreover, upon aerating for 24 hours, only partial recovery was found to have been made. The minimum partial pressure of oxygen for root-growth in the sour orange appears to lie between 0.6 and 1.6 per cent, but the experiments did not indicate it more closely.

Rough Lemon.

Some experiments on the rough lemon were carried out with 1, 2, and 3 per cent oxygen, with nitrogen and at various soil temperatures. In the introductory tests, as, in fact, in all cases, the rate of growth was first observed in normal soil-air and before the gaseous mixtures were given, and also for comparative purposes after aeration between each test. The first experiment was begun August 3, when 3 cultures were studied. Of these No. 1 was at 27° and the others at 18° C. In normal soil-air the root-growth was as follows: No. 1, 3.5, and Nos. 2 and 3, 2 mm. in 24 hours. Then 2 per cent oxygen was given each of the cultures for 24 hours, during which time no root-growth took place in any of the plants. They were then aerated for 24 hours, during which time the growth was 3.5, 1.5, and 0.5 mm. for each, respectively. After this, 2.8 per cent oxygen was given for a day, when the following root-growth was noted: 2.5, 0, and 0.5 mm., for the same plants. Following this treatment, the cultures were again aerated, when the growth for 24 hours was found to be 4.5, 1, and 1 mm. Finally, 2.8 per cent oxygen was again given for 24 hours, when the growth became 2, 1, and 1 mm. for the cultures.

The results of the preliminary test with the rough lemon can be summarized as follows: Roots at 18° and at 27° C. stop growth when a soil atmosphere containing 2 per cent oxygen is given. When, however, the amount of oxygen is increased to 2.8 per cent, a certain amount of root-growth goes on at both temperatures, but most rapidly at 27° C.

It perhaps should be stated that oxygen determinations were made of each tube every day, and, as usual, the amount of oxygen in each tube was peculiar to itself; that is, there was found to be as much as 0.4 per cent difference in the oxygen-content of tubes drawing from the same gasometer. This, as explained before, is due in part to inward diffusion of oxygen from the atmospheric air, and in part and in the other direction, to the consumption of oxygen by the roots themselves. In the experiments just described, the first analyses of the cultures at the highest temperatures were usually or always slightly under those of the cultures running at the lower temperature.

On August 15 an experiment was started with 5 cultures of the rough lemon and 1 of Golden Bantam sweet corn, for comparison. The main results are summarized in table 13.

The oxygen determinations were somewhat inconsistent, as had been found in the earlier experiments. Thus, in the corn, the oxygen-content of the tube was actually about 0.4 per cent higher than in the gasometer, that of the lemon tubes at the lower temperature was about the same, and at 30° it was slightly higher. But the differences, so far as the latter are concerned at any rate, were not sufficiently large to vitiate the experiments, but they pointed, in some instances at least, to active absorption of oxygen on the part of the root.

TABLE 13.—*Root-growth of rough lemon and Golden Bantam sweet corn in a deficiency of oxygen (24-hour periods, in millimeters).*

Experimental atmosphere.	Corn	Lemon.		
	21.5° C.	30° C.	22° C.	18° C.
Aerated	17.5	5 4.5	2	0.5
1 per cent O_2	1	0 0	0	0
Aerated	7	2 2	2.5	0.5
1.2 per cent O_2	3.5	1 0	0.5	?
Aerated	8.5	2 3	2	?

The general results on species of *Citrus* can be briefly given. Growth of roots appears to go on in the sweet orange, although not rapidly, in 75 per cent or over carbon dioxid. This indicates that they are highly tolerant to a great excess of the gas.

When the amount of carbon dioxid present did not exceed 21 per cent, the root of sweet orange was found to grow when the quantity of oxygen present was less than 2 per cent.

In the total absence of carbon dioxid, root-growth of sweet orange and lemon is apparently inhibited in a gaseous mixture containing about 2 per cent, but it continues in about 2.5 per cent oxygen. The minimum in the Brazilian sour orange, however, is apparently lower than in the sweet orange. Root-growth stops in the total absence of oxygen.

Covillea tridentata, Krameria canescens, Mesembryanthemum æquilaterale, and Prosopis velutina.

Two experiments were run in which seedlings of *Covillea, Krameria,* and *Prosopis,* and cuttings of *Mesembryanthemum* were used. In these, 25, 42, and 75 per cent carbon dioxid, with atmospheric air, were employed, by which the actual amount of oxygen present varied between 5 and 15 per cent. The possible effects noted were, therefore, in large degree or wholly, to be associated with the carbon dioxid itself. In these experiments the cultures were exposed freely to the temperature changes of the room in which they were carried on and the root-growth was determined, by means of a horizontal microscope. The observation periods were accordingly frequent and the duration of the experiment relatively short.

In the first experiment the gaseous mixture contained 75 per cent carbon dioxid. *Covillea, Krameria,* and *Mesembryanthemum* were observed. The first reading was made at 11 o'clock and, except for the first and second hours, were repeated at 2-hour intervals throughout the day. From 11 a. m. until 12 o'clock noon, the roots both of *Covillea* and of *Mesembryanthemum* increased 0.2 mm. in length, while that of *Krameria* decreased in length the same amount. During the second hour the roots of all three species grew 2 mm. At the end of the third hour the root of *Covillea* was

found to have decreased 0.2 mm. in length, while the roots of *Krameria* and *Mesembryanthemum* were about 0.1 mm. longer. During the first 3 hours in 75 per cent carbon dioxid, therefore, the net increase of *Covillea* was 0.2 mm.; of *Mesembryanthemum*, 0.5 mm.; and of *Krameria*, 0.1 mm. Thereupon all of the cultures were aerated and were left in normal soil-air until 9 o'clock of the following day. At 4 o'clock, or 2 hours after the beginning of the aeration, the following condition was noted: The root of *Mesembryanthemum* had not grown, but that of *Krameria* had increased 0.2 mm. and that of *Covillea* 0.5 mm. in length. At 6 o'clock, that is, for the third-fourth hour period following aerating, the root of *Covillea* had not changed, but that of *Mesembryanthemum* had grown 0.1 mm. and that of *Krameria* 2 mm. At 9 o'clock on the following morning it was found that all of the roots had grown somewhat. Thus, the growth in the case of *Covillea* had been 0.3 mm.; of *Mesembryanthemum*, 1.5 mm.; and of *Krameria*, 3.1 mm. The day temperature of the laboratory was 25° to 29° C. and the minimum night temperature about 12° C.

In the second experiment the effect on the rate of root-growth of 42 and 25 per cent carbon dioxid was observed. The gaseous mixture was given 2-hour periods, followed by aeration. The general course of the experiment was the same as before. At $7^h\ 30^m$, 42 per cent carbon dioxid was given cultures of *Covillea*, *Mesembryanthemum*, and *Prosopis*. At $9^h\ 20^m$ it was found that the root of *Covillea* had increased 0.3 mm. in length, that of *Mesembryanthemum* 1.3 mm., and that of *Prosopis* 0.6 mm. The cultures were then aerated and left in normal soil-air for 3 hours and 45 minutes, during which time the root-growth was as follows: *Covillea*, 0.8 mm.; *Prosopis*, 0.1 mm.; and *Mesembryanthemum*, 1.4 mm.

At $7^h\ 20^m$ on the following morning 25 per cent carbon dioxid was given the same three plants and the gas administration was continued until $9^h\ 20^m$. While in the mixture the root of *Covillea* made no growth, that of *Prosopis* grew 0.6 mm., and that of *Mesembryanthemum* 1.1 mm. The cultures were then aerated and left under observation while in normal soil-air for over 7 hours. At the end of this time the root of *Mesembryanthemum* was seen to have increased 6.5 mm. in length, that of *Prosopis* 3.3 mm., and that of *Covillea* 0.1 mm.

It will be seen that the short-time experiments with an excessive amount of carbon dioxid do not give consistent results. In 75 per cent the rate may be momentarily accelerated, or shortening of the root may take place. But in the case of *Covillea*, as was observed in other tests, an excess of carbon dioxid seriously affects the rate of root-growth, and possibly when long continued a relatively small percentage would bring about its cessation.

Cucumis melo.

A single series of experiments was run on a variety of the cultivated muskmelon, in which the effect on the rate of root-growth of a soil atmosphere poor in oxygen was under observation. The gaseous mixtures employed contained 0.5, 7, and 10 per cent oxygen, with the diluting gas nitrogen.

In the experiment with 7 per cent oxygen, 2 cultures were used and the period of gas administration was 4 days. At the beginning the tap-root of culture No. 1 was 5.5 cm. in length and that of No. 2 was 4 cm. In No. 1, where 3 laterals in addition to the tap-root were measured, the root-growth was as follows: For each 24-hour period of the 4 days the increase in length of the main root was 13, 12, 6, and 3 mm. During the same time the 3 laterals grew from 3 to 6.5 mm., the daily amount becoming gradually less, as in the case of the tap-root.

In culture No. 2, with 7 per cent oxygen, the tap-root grew 10 mm. daily for each of the first two 24-hour periods and then growth ceased, and essentially the same results were obtained in the case of a third culture.

So far as the rate of growth in 10 per cent oxygen is concerned, it was found that a fairly rapid rate occurred during the 4-day gas administration. The total amount was 35 mm., or about what was found to occur when 7 per cent oxygen was employed.

At the expiration of the experiment last referred to, 0.5 per cent oxygen was used for a period of 24 hours and growth of all roots ceased.

An experiment was then set up in which 0.5 per cent oxygen was used in connection with fresh cultures. In this test, which was run 72 hours, the temperature conditions were the same as before. Cultures Nos. 4 and 5 were used. At the beginning of the experiment the main root of No. 4 was 7.5 cm. long and that of No. 5 was 6 cm. The daily root-growth of the tap-root while in experimental atmosphere was as follows: in No. 4 it was 3, 2.5, and 0 mm., and in No. 5 it was 3, 4, and 2 mm.

It should be pointed out that the work with the muskmelon, although not extensive, appears, however, to indicate that the species is probably dependent on a fairly abundant supply of oxygen in the soil. There is probably no difference, so far as the experiments revealed this, as to the effect on the growth-rate of 7 and 10 per cent oxygen, although the possibility is in no way excluded that in case of relatively high temperatures, especially of the soil, a differential effect might be demonstrated as between gaseous mixtures of these two compositions. So far as the daily diminution in the growth-rate is concerned, it may well be that the experiments in all instances chanced to coincide with the expected slowing of the rate of growth which is normally associated with the grand period of growth and with the formation and growth of laterals as determined by Priestly and Evershed,[1] and which, as shown in this study, may be seen in cotton also.

Cupressus macrocarpa.

A single series of experiments was run on the Monterey cypress, whose chief interest lies in the extremely restricted distribution of the species. Young plants, about 12 months from the seed, were used. The experiments were with 1 per cent oxygen, diluted with nitrogen and with normal soil-atmosphere. The temperature of the soil, during the experiments, was kept between 21° and 23° C.

Two cultures were used. At the beginning of the experiments, and before the gaseous mixture was given, the rate of root-growth in normal soil-air was noted for three consecutive days. This, for No. 1, was found to be 3, 2.5, and 2.5 mm. for each 24-hour period, and for culture No. 2 it was 4.5, 2.5, and 3.5 mm. for the same periods.

On the fourth day and to the eighth day the experimental mixture was administered continuously. In culture No. 1 the root increased 1 mm. in length the first 24 hours. During the second day the root-tip turned sharply from the inner wall of the tube and was lost to sight, but at the end of the third day it reappeared and remained in view until after the sixth day, when the experiment was concluded. On the second, third, fourth, and fifth days the growth was 0.5 mm. each day. But no growth took place on the sixth day.

The history of culture No. 2 was in all essentials the same as that of No. 1, except that during the first day in the experimental mixture the root grew 2 mm. Growth terminated the sixth day.

[1] Growth Studies. I: A quantitative study of the growth of roots. Ann. Bot., vol. 36, p. 225, 1922.

Both cultures were aerated on the seventh day of the experiment and were left under observation in normal atmospheric air for 3 days. In culture No. 1, root-growth took place the first day in normal atmosphere, but none subsequently. In No. 2, a growth of 1, 1, and 1.5 mm. for each of these days was noted.

It would appear to be possible, therefore, that the roots of relatively young seedlings of the Monterey cypress are not especially sensitive to a marked deficiency of oxygen.

Echinochloa crus-galli.

The reaction of the roots of *Echinochloa* to differences in the aeration of the soil was observed in two series of experiments, in which 0.5 per cent oxygen, balance nitrogen, and 50 per cent carbon dioxid with atmospheric air, were employed. Seedlings were used of which the roots were between 12 and 20 cm. in length.

Six cultures were used in the oxygen series. They were subjected to parallel treatment so far as possible. At the beginning of the series, and while the roots were in normal soil-air, a root-growth of from 2 to 4.5 mm. was demonstrated in each culture for each of the two 24-hour periods.

The gaseous mixture deficient in oxygen was then given during 5 days continuously and the root-growth was noted each day. At the end of the first 24 hours, growth of 1 and 2 mm. took place in two cultures, but none in the others. In culture No. 5 growth ceased with the beginning of the gas administration and was not resumed. In No. 4, 0.5 mm. growth occurred the first day and none afterward. In No. 2, the root bent sharply but continued to increase in length at about 0.5 mm. each day, during 4 days and then growth ceased. Culture No. 3 showed a growth of 2 mm. the first day, 1 mm. the second day, and none after that. In cultures Nos. 1 and 6, however, there was from 1.5 to 4 mm. growth during each of 4 days, none the fifth day in No. 1 and less than 1 mm. in No. 6. The root tip of No. 6 turned sharply away from its earlier direction of growth during the last day or two of the test.

The reaction of the roots of *Echinochloa* to an excess of carbon dioxid was observed in 4 cultures, which were kept at a temperature of 27° C. During 24 hours just preceding the gas administration, the roots increased from 3.5 to 8.5 mm. in length. Immediately thereafter the mixture was given continuously 48 hours and observations were made each day. At the end of the first 24-hour period it was seen that the root of No. 2 had grown 1 mm. and that of No. 5 had grown 5 mm. but that there was no growth in the other two cultures. There was no root-growth in any of the cultures during the last day.

At the close of the experiment the cultures were aerated and placed under observation for 48 hours while the roots were in normal soil-air. No further root-growth took place.

It should be remarked that each of the cultures of the carbon-dioxid series was irrigated each day by drawing water through the soil of the tube. To do this the connection with the gasometer was broken and a certain amount of atmospheric air entered the soil, but the experimental mixture was immediately run through as at the beginning of the experiment. In the case of the oxygen series, on the other hand, the connection with the gasometer remained intact and irrigation was accomplished by up-ending the tubes. In such case no atmospheric air entered.

From the general results of the experiments it will be seen, therefore, that *Echinochloa* should probably be classed among the species which require good conditions of soil aeration.

Eriogonum nudum.

Eriogonum nudum occurs at Carmel on high land and in sandy soil. In the aeration studies, transplanted specimens were used and were found to make very good growth in the culture tubes. Two series of experiments were carried on, of which the first had to do with a deficiency of oxygen and the other with an excess of carbon dioxid in the soil. In the first, gaseous mixtures containing 0.5 and 2 per cent oxygen, with nitrogen, were used, and in the second, 10 to 25 per cent carbon dioxid with atmospheric air. The temperature of the soil was between 22.5° and 27° C.

In the first experiment with a deficiency of oxygen, in which 2 per cent was used, the culture was under observation 72 hours. In normal soil air, and for 24 hours at the beginning of the experiment, a root-growth of 15 mm. was noted. The culture was then given the gas for 48 hours continuously, during which time a growth of 33 mm. occurred, of which 15 mm. was the first 24 hours. At the end of the 48-hour period the root-tip turned sharply from the wall of the tube and was lost to view, but it was learned, by removing the soil, that no growth took place subsequent to the last observation.

In the second and third experiments of this series 0.5 per cent oxygen was used. The experiments lasted for 3 and 4 days with similar results. In normal soil-air and for 24 hours preceding the gas administration a growth of 4 and 5 cm. occurred. The gas was then given for 48 and 72 hours. In the first culture 1 mm. root-growth took place during the first 24 hours and none afterward, and in culture No. 2 growth of the root stopped immediately upon the administration and was not renewed.

The second series with carbon dioxid was begun with an observation of root-growth in 10 per cent of that gas. A single culture was used. At the beginning of the test the root studied was 32 cm. long. For a 24-hour period, while in normal soil-air, the root increased 6.5 mm. in length. It was then given 10 per cent carbon dioxid for 5 days with the following measurements for the growth of each day: 3, 2, 0.5, 0, and 0 mm. This specimen came into flower during the experiment.

The second experiment of the series was run for 17 days, during which a gaseous mixture containing 25 per cent carbon dioxid was given continuously. In normal soil-air for 24 hours at the beginning of the experiment the root grew 3.5 mm. and the subsequent daily growth was as follows: 2, 1, 0, 0.5, 0.5, and 0 mm. and growth was not renewed.

It appears, therefore, that so far as the experiments indicate the aeration relation of the roots of *Erigonum*, the species is not tolerant of a great deficiency of oxygen or a great excess of carbon dioxid in the atmosphere of the soil.

Fouquieria splendens.

Fouquieria splendens occurs in southern Arizona to a large extent, if not exclusively, in soils which are well aerated and possibly also relatively warm. The roots are largely superficial. Certain experiments which were carried out on the relations of the growth of the roots of the species to the temperature of the soil indicated that the optimum for growth is fairly high. For example, the following records of root-growth for 12-hour periods, at the temperatures given, appear to indicate this. At 14.5° C. the growth was 4 mm., at 26.5° C. it was 8.5 mm., and at 31° C. it was 11.6 mm. At the best, however, the rate falls considerably below that for seedling *Prosopis*, in which, for the same time periods and temperature, the root-growth was seen to be 6, 24, and 27 mm. The rate of growth in *Fouquieria* was observed in cuttings and that in *Prosopis* in seedlings, which, however,

were relatively old. The growth-rate in *Fouquieria* most nearly approaches that in *Opuntia versicolor*, but it is possible that the minimum may be somewhat lower than in that species.

The specimens used in the experiments were dug up on the grounds of the Desert Laboratory and either were without branches, or bore one or two only. The shoots were about 15 cm. in length. In removing the plants from the soil the roots were of necessity badly injured, so that new ones were formed after the plants had been transplanted into the tubes. Fresh leaves also were formed at the favorable temperature, 27° C., used. After root-growth was seen to have begun the plants were prepared as usual and an experiment was set up which lasted 6 days. Two per cent oxygen, with nitrogen, was given the cultures continuously; as a result it was seen that root-growth occurred each day, although the rate was relatively slow.

The results, although thus meager, are, however, of interest and point to the probability that *Fouquieria* requires a well-aerated soil for growth of the roots. It will be noted that the percentage of oxygen used was fairly high, but that the temperature of the soil was about 4° C. below that at which the most rapid root-growth occurs. It seems possible, therefore, that had the soil temperature been at optimum, with the amount of oxygen remaining 2 per cent, other results would have occurred, namely, root-growth would have ceased.

Gossypium barbadense var. pima.

Numerous experiments were carried out on the oxygen relation of the Pima variety of *Gossypium barbadense*, the seed for which was furnished by the Arizona Experiment Station.

Seedlings only were studied. In the early stages of development the main root usually becomes several centimeters in length before the emergence of the young shoot and before the cotyledonary leaves become clear of the seed-coats. The latter develop relatively slowly and the hypocotyl may be from 5 to 10 cm. in length before they attain full size. In the material used the cotyledonary leaves each get to measure about 3 by 5 cm. before the plumule appears. They thus are important organs for photosynthesis. It is usually relatively late before the first laterals make their appearance, and also the main root is usually the most important member of the root system of the seedling. In many instances, however, the sequence of development of the organs of the seedling and their relative importance was seen to be other than that above sketched. Not infrequently, and especially during cold and foggy weather, it was found that the main root made little growth, but that the shoot was the first to undergo development, to be followed by the formation of laterals, and possibly later by the elongation of the tap-root.

Under normal conditions of soil aeration it was found that the daily root elongation for the temperatures used varied from about 9 to about 52 mm., or more, as shown in table 26.[1] It there appears that a slow growth rate occurs at 17.5° and at 16°, and that the rate rapidly increases with rise in temperature, with the effect that rapid growth occurs over a relatively wide temperature range, as from 22° to about 30°, when it becomes less and falls rapidly at about 35° C. The optimum temperature for root-growth in the variety of cotton used is apparently not far from 25° C. However, the most rapid daily growth was noted at 30°. An additional characteristic was seen to lie in the differences in the smoothness of the curve of daily root elongation as between the different temperatures used. Thus, at 21.5° or 22°, out of 10 observations the range of daily growth was

[1] Page 156.

seen to lie between 20.5 and 32 mm. At 30° it was between 3 and 52 mm. These measurements are of the main root only. Observations on the laterals indicate that the daily growth usually does not exceed 5 mm. and frequently is not over 2 mm.

The wide daily variation in the elongation of the main root at constant soil-temperature is in part to be related to the stage of its development. From the first emergence from the seed to the culmination of the "grand period" the rate accelerates, at first gradually and finally rapidly, until a maximum is reached, after which it becomes gradually less for about 4 days, when it appears to be fairly stabilized. In the last condition growth variation is occasioned by external forces for the most part, such as the temperature of the soil.

Root-growth in seedling *Gossypium* affords a good illustration of the relation between the development of laterals and the decrease in rate in the "grand period." It is now known that growth of a root can be represented by a series of S-curves, by which growth acceleration followed by growth decrease is indicated, and that the latter occurs at the time rootlets are organized.[1] This feature was investigated in the cotton in which observations were made at a soil temperature of 21.5°. The growth was determined in part by measuring the rate of daily elongation and in part by computing the root-mass. The following will illustrate the daily growth of the main root of a typical seedling at the temperature above given. During studies covering 11 days the daily growth was as follows: 15, 20.5, 22.5, 22, 28, 32, 31.5, 32, 21.5, 22, and 22 mm. Up to the fifth day no laterals were to be seen, but on that day, with a growth 28 mm., translucent spots appeared at many places on the main root, which became on the following day as many laterals. On the sixth day about 16 rootlets were seen to have emerged. During the seventh and eighth days growth was about 32 mm. daily; rootlets appeared in large numbers. About 75 were formed up to the eighth day, the combined daily elongation of which was 250 mm., more or less, or approximately 10 times that of the parent root. On the last day of the observations the volume of the laterals and of the main root was determined. It was found that the volume of the laterals was about 525 c. mm., and that the volume of that portion of the main root which was organized at the same time was about the same. When plotted as a curve, therefore, the growth of the main root at first rises and then falls, as would be expected during the course of the "grand period," but when the growth of the root system taken altogether is plotted as a curve, the course is a fairly consistent ascending one.

The concentration of oxygen used in the experiments varied from 0.5 to about 8 per cent and the temperatures employed were mostly between 20° and 30° C., although experiments were conducted below the former and above the latter. The course of typical experiments only will be given in some detail, and that of the balance will be briefly characterized.

On May 2, an experiment was set up in which 4 seedlings were used. Laterals were present on each and were relatively well developed in No. 1. Nos. 1 and 2 were in a thermostat running at 30°, and the balance were kept at 20° C. During a 24-hour period just preceding the gas administration the main root in No. 1 increased 26.5 mm. and that of No. 2 increased 45 mm. in length. During the same time the root of No. 3 grew 9 mm., and that of No. 4, 10.5 mm. in length. The soil of all of the tubes was well aerated at this time.

[1] J. H. Priestly and A. F. C. H. Evershed. Growth studies. I: Quantitative study of the growth of roots. Ann. Bot., **36**: 239, 1922.

Beginning on May 3, all of the roots were given 8 per cent oxygen for 48 hours continuously. The root of No. 1, at 30° C., increased 25.5 and 25 mm. on the 2 days, and that of No. 2, 31.5 and 22 mm. At the same time the main root of No. 3 grew 7.5 and 11 mm. and that of No. 4 grew 8.5 and 10 mm. The latter were at 20° C. It will appear, therefore, that root-growth in 8 per cent oxygen, and both at 20° and at 30° C., is about the same as in normal soil air.

On May 5 an additional experiment was run at a temperature of 35° C. Two plants were used, the roots of which grew 17 and 11.5 mm. in 24 hours and while in normal soil-air. They were then given 8 per cent oxygen for 24 hours. The growth fell to 5.5 in the first plant and was 10 mm. in the second.

An experiment with 6 plants was begun April 29 with 4 per cent oxygen. Two plants each were at 30°, 25°, and 21° C. temperature throughout. The initial length of the roots varied from 10.5 to 13.5 cm. In two, No. 11 and No. 16, laterals were beginning to form when the experiment started. It will be sufficient to mention the behavior of one plant only at each temperature used. The root of No. 9, at 30°, increased 32 mm. in normal soil-air during 24 hours preceding the use of the gaseous mixture. It was then given 4 per cent oxygen for 24 hours and the growth dropped to 6.5 mm. The culture was then aerated and left an additional 24-hour period under observation while in normal soil-air. During this time the root increased 15.5 mm. in length. No. 11, at 25° C., grew 9 mm. while in normal soil for 24 hours, but when given the gaseous mixture for an equal period the growth became 3.5 mm. Upon being aerated, however, the growth in 24 hours increased to 5.5 mm. No. 14, which was kept at 21° C. and which like No. 11 was beginning to form laterals, grew 18 mm. in normal air during one day at the beginning of the experiment. In 4 per cent oxygen for 24 hours the growth was seen to be 15 mm. After being aerated the root increased 11.5 mm. in length during the final observation period.

It will be seen that the relative growth of the root of the cotton in 4 per cent and in 8 per cent oxygen is high, for the most part, but that at the higher temperatures it is less than at lower temperatures. Up to and including a temperature of 30° it is evident, from the experiments quoted as from other observations not given, that about 8 per cent oxygen is sufficient for a "normal" rate of growth, but that at 35° the ratio of growth in normal soil-air to growth in 8 per cent oxygen is less than 1. Further, while a normal rate of root-growth may take place in 4 per cent oxygen when the temperature is about 21°, the growth-ratio falls with increasing temperature until it may be about 0.23. Thus, it might be expected that at 35° C. growth would cease in a soil atmosphere with 4 per cent oxygen.

In concentrations of oxygen between 2 and 3 per cent the results were somewhat various. In 3 instances a normal rate of root-growth was observed to occur, while the root was in about 2.5 per cent oxygen, at 20°, 24°, and 30° C., but, as will be discussed somewhat further on, while the rate might usually be normal at the lowest temperature mentioned, other factors than temperature may have come in to influence growth where the temperature was higher. Out of 17 experiments in which between 2 and 3 per cent oxygen was used, and at temperatures ranging from 17° to 35° C., in only 4 instances was it seen that a normal rate of growth occurred, and of these, in addition to those already mentioned, one was at a soil temperature of 19° C. No growth was seen to take place at 35°. At 30° the growth-ratio varied from 0.06 to 0.27. At 24° the extremes of the ratio of growth were 0.26 and 0.55, with the single exception above given, and

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at 20° the ratio lay between about 0.66 and 1. Although, therefore, not a little variation was found in the rate of root-growth in a gaseous mixture containing from 2 to 3 per cent oxygen, and this was true at all except the highest temperature used, it is apparent that at the lower temperatures the rate of growth is highest and may become normal around 20° C.

From the results of experiments on root-growth in more than 2 per cent oxygen, therefore, it is evident that about 2 per cent is the least concentration at which a normal rate of growth will occur at any temperature suitable for root-growth, and that a normal rate will be expected to occur in about 8 per cent when the temperature is increased to about 30°, or possibly somewhat higher. At 35°, however, the rate rapidly decreases. However, a normal rate at the latter temperature may go on in about 10 per cent oxygen. However this may be, it is clear that the range of the upper critical concentration lies between 2 to 3 and 8 per cent or above, depending on the temperature at which the tests are made. The range of this cardinal concentration, therefore, probably exceeds 5 per cent.

Where the concentration of oxygen is below 2 per cent it is doubtful if normal growth takes place, even at the low temperatures used. Such percentage, therefore, marks the approximate upper boundary of the lower critical concentration of oxygen for root-growth in the cotton.

Tests with 0.6 per cent oxygen show that growth of the main root does not take place at any of the temperatures used, that is, between about 16° and 35° C. Neither was it possible to induce growth when the amount of oxygen was raised to 1 per cent, although such is recognized as a possibility under favorable conditions, as with low temperatures. At 1.2 per cent oxygen, however, no growth was seen to occur at 35°, and doubtfully at 30°, but at 24° a growth-ratio of 0.14 and at 20° a ratio of 0.3 was obtained. About 1.2 per cent oxygen, therefore, is the lower critical concentration of oxygen for root-growth in the cotton studied. It will be seen from this that the range of the lower critical concentration is little, if any, more than 1 per cent for temperatures up to 30° C., at least.

It should be remarked in passing that whether, or to what possible degree, external factors other than temperature modify the effects on root-growth of so small concentration of oxygen is not known. If they do, too narrow interpretation of such results as those given should not be made. It is believed, however, from over 100 tests on the Pima variety of *G. barbadense*, that the results presented represent the essential facts. However, attention should be called here to a condition which is discussed in the final section of the study, which might modify the conclusions as given for roots growing in a small amount of oxygen, that is, less than 1 per cent. It was often noted in experiments on cotton, and on other species as well, that when the percentage of oxygen was so small as 1 per cent, or less, the analyses showed a larger amount present than was in the reservoir. This frequently was more than 1 per cent higher than the experimental mixture being used. In *Allium* and *Potentilla*, as mentioned elsewhere, such inward diffusion was shown to occur to a large extent, at least, through the enveloping bracts, scales, or leaf-bases, but in other species, as in cotton, where such were absent no such explanation seemed to hold. It was thereupon supposed to lie in experimental error of some kind, such as diffusion through rubber connections, or through the seal about the base of the plant.

Although the main connections were already of fairly heavy rubber and tightly wound with wire, pressure tubing was substituted so far as possible, and extra precaution was taken to make the plasticine seal unusually heavy and to be especially well packed about the stem. In a very large

proportion of the tests, however, the larger percentage of oxygen was still to be found. At length blank tubes were included with the tests, and were used alternately with the cultures proper. In these special experiments 0.6 to 0.8 per cent oxygen was used. Two or three of the tests will suffice to show representative results. On August 30 a test was begun in which cotton plants numbered 94 and 99 were used, together with two blank tubes. All were connected so that they were given the same gaseous mixture, namely, 0.6 per cent oxygen, with nitrogen. No. 94 and one blank tube were in the thermostat running at 23.5° and the other tubes were kept at 19° C. The oxygen tests showed from 0.6 to 0.8 per cent oxygen in each tube. The experiment was allowed to run 48 hours without being disturbed. At the end of this time 2.8 per cent oxygen was found in the culture tube carrying No. 94 and 1.6 per cent in the blank control, and 2.4 per cent oxygen in No. 99 with 1.6 per cent in its associated control.

The cultures were given water and were resealed and allowed to remain an additional 48 hours in the same gaseous mixture. At the outset, 0.8 per cent oxygen was found in each tube. At the end of the second period of 48 hours, 2.4 per cent oxygen was in the tube of No. 94, with 0.8 per cent in the control and 2 per cent in each of the other tubes. Thus, in this instance, No. 99 did not show any difference in oxygen-content from the blank control. One more culture, with another control, was then added and the test was continued about 2 more days. During these days, however, oxygen determinations were made at $6^h\ 30^m$ in the morning, and 5^h in late afternoon. There were 5 successive oxygen determinations of which three were in late afternoon and two were in early morning. The possible effects of photosynthesis were thought in this manner to be detected.

The following summarizes the leading results: In No. 100, temperature 30° C., in every instance there was found to be more oxygen in the tube with the plant than in the control without a plant; the same was true of 94, except that in one reading both control and culture were alike. In No. 99, in which the last reading of the last 48-hour test was the same both in the control and in the culture, the oxygen percentage was the same in both and varied only from 0.8 to 1 per cent during the 5 successive determinations in question. The tests did not show consistent difference in oxygen-content of the culture tubes between the two daily readings. In No. 100 there was a slightly larger percentage in the morning throughout, but neither of the other cultures were consistent in this regard. The highest reading for No. 94, however, was in early morning.

A series of experiments was run in which the cultures and the blank controls were kept continuously in darkness for 48 hours at different soil temperatures. It is not necessary to describe the results further than to say that both blank controls and tubes bearing plants at the beginning or at the end contained about the same percentage of oxygen. The concentration of oxygen at the outset was 0.6 to 0.8 per cent and at the end of the period it was from 1.2 to about 2 per cent, showing a considerable inward diffusion, in which, however, as remarked above, cultures and controls showed no difference. No root-growth took place in any of the cultures referred to in the preceding summary of experiments on possible oxygen translocation.

Gossypium hirsutum.

Some aeration experiments were carried out on seedlings of variety *acala* of *Gossypium hirsutum*, the seed of which was provided by the Arizona Agricultural Experiment Station, Tucson. Parallel with the experiments

on cotton, and for the purposes of comparison, two cultures of willow were under observation. In these experiments daily oxygen determinations were made by drawing off small amounts of the experimental soil-atmosphere from the cultures which was checked by analyses of the mixture in he gasometers.

Four parallel cultures were run. Of these, two were kept in a thermostat at 27.5° C., during the first part of the experiment, and were then transferred to a thermostat at 22° C., and two cultures were kept at 22° C. at first and were later placed in the thermostat running at 18° C.

At the beginning the roots of the seedlings were 6, 6.5, 7, and 10 mm. in length.

In normal soil-air for 24 hours the roots of 2 of the cultures, which had been in the thermostat at 27.5° C., increased 7 and 15 mm. in length, while those of two other cultures, at 22° C., grew about 3 mm. only.

A mixture consisting of 1.2 per cent oxygen, with nitrogen, was then given all of the cultures for 24 hours. At the expiration of this time it was found that no growth of the roots had taken place.

In the analyses for oxygen, which were made of the cultures in the deficiency of oxygen, several samples were drawn, and it was found that in each instance the first had about 0.4 per cent less oxygen than was in the gasometer, but that the latter samples contained the expected amount.

The cultures were then aerated, but not irrigated, and while in normal soil-air for 24 hours the following amounts of growth were determined for the roots: 3.5, 5.5, 2.5, and 0 mm.

At the close of this period all of the cultures were irrigated and were placed in thermostats, which were kept at the lower temperatures already mentioned, 22° C. Under these conditions the growth for 24 hours was 6, 6, 5, and 9 mm.

Following the observation last referred to on root-growth in normal soil-air, a gaseous mixture containing 1.6 per cent oxygen was given for 24 hours, and it was found that no root-growth in any of the cultures took place during this time.

Oxygen analyses were made at the expiration of the last test, and it was found as before that the experimental atmosphere which was immediately about the roots was poorer in oxygen than the mixture in the gasometer, and also that it contained less than the third and fourth samples drawn from the tube.

During a portion of the experiment on cotton, and as a control, some willow cuttings were under observation. Growth of the roots was demonstrated in these while in normal soil-air. They were then given 1.6 per cent oxygen for 24 hours and it was found that while the rate of growth was less than in normal soil-air, relatively rapid growth nevertheless took place. It appears clearly shown that the two species react differently to the same deficiency of oxygen, and that the cotton seedling is more sensitive in this regard than the cutting of the willow.

Analyses were made of the experimental soil atmosphere which was in the willow culture at the end of the 24-hour experiment last referred to. The results were quite different from those on the cotton. No analysis showed less oxygen present than was in the gasometer.

Hordeum sativum.

The seeds of the barley studied were obtained in the open market and were planted directly in the tubes, thus avoiding transplanting. In addition to observations on root-growth in normal soil-air, the aeration experiments

included the use of the following gaseous mixtures, namely, 0.5, 1, 2 per cent oxygen, with nitrogen, and cylinder carbon dioxid. The temperatures employed were between 23° and 27° C.

Numerous measurements on root-growth in normal soil-air showed that at a soil temperature of 27° C., it varied from 10 to 29 mm. in 24 hours.

The behavior of culture No. 2 of the oxygen series will sufficiently well illustrate the reaction of the roots to 2 per cent oxygen. In this case growth during the 24-hour period immediately preceding the gas administration was found to be 21 mm. The culture was then given the mixture for 48 hours continuously and the gas was static. Two roots of the culture were under observation and it was found that they increased 16 and 18 mm. during each 24 hours while in the experimental atmosphere.

In a second experiment, also with 2 per cent oxygen and which also was for 48 hours, the gaseous mixture was changed several times, so that in fact it amounted to slow streaming. The temperature of the soil was as before. In this case, where No. 2 was used, the daily growth of the root was 18 and 28 mm.

Finally, the culture was given static 2 per cent oxygen for 96 hours continuously. Two roots were under observation in the culture. Of these, No. 1 grew 19 mm. in 96 hours, while root No. 2 increased 10 mm. in 72 hours. The roots had every outward indication of normalcy, and gave no indication by bending away from its course of possible loss of the geotropic response.

A second series with oxygen was run with 6 cultures, in which 0.5 and 1 per cent oxygen were employed. Before the gas administration a daily growth of the roots from 15 to 25 mm. was demonstrated while they were yet in normal soil-air.

The cultures were given 1 per cent oxygen continuously for 13 days. In this experiment the tubes were not opened for the purpose of irrigating, but were up-ended each day, or as often as required. The mixture was under constant and slight pressure and, except as noted below, was static. Owing to the great variation in the rate of the growth of the roots, the history of each culture, so far as necessary, will be given.

The root-growth of No. 1 in normal soil-air and for 24 hours immediately preceding the gas administration was 25 mm. Then 1 per cent oxygen was given for 10 days and the growth for each 24 hours was measured. This was found to be as follows: 1, 9, 15, 11.5, 22, 8.5, 14, 10.5, 5, and 4.5 mm.

In culture No. 2 the growth in normal soil-air for 24 hours was 23 mm., and the daily growth in the 1 per cent oxygen was 10, 18, 2.5, 10, 11.5, 9, 2.5, 2, 0, and 0 mm. During the first part of the experiment with No. 2 there was a slow outward gas leak, revealing the fact that the mixture was slowly streaming through the culture. This was stopped and the mixture was static for the balance of the period.

Root-growth of culture No. 3 for 24 hours, in normal soil-air and immediately preceding the gas administration, was seen to be 15 mm. Then 1 per cent oxygen was given the culture. At the end of the first 24-hour period the root was seen to have increased 2.5 mm. in length and the tip was found to have turned away from the wall of the tube. It was hidden by the soil and was not seen subsequently. On the seventh day a second root appeared and its growth was observed. During the balance of the experiment the daily growth of this root was 11.5, 11, 13, 14, 5, and 8 mm.

Culture No. 4 had a root-growth of 23 mm. while in normal soil-air for 24 hours; when, however, it was given 1 per cent oxygen, the daily

rate immediately fell, as the following measurements indicate: 6.5, 6.5, 13, 9, 10.5, 10, 10, 11, 14, 9, 8, and 7 mm. In this experiment the increase in the rate of growth for a portion of the experimental period was not explained.

The history of the root-growth of No. 5 was substantially as of No. 4. In the case of No. 6, however, which had an initial growth while in normal soil-air of 17 mm. the root remained in view only 3 days in the gaseous mixture and then turned away from the wall of the tube and was lost to view during the balance of the experiment.

The leading result of the experiment with 1 per cent oxygen will be seen to be an indication that the species may be relatively insensitive to a marked oxygen deficiency. In order to test this somewhat more closely, the same cultures were given 0.5 per cent oxygen for a period of 9 days immediately following the experiments above outlined. The results were various. No growth occurred in Nos. 1, 2, and 6. In each of the other cultures two roots were observed. In culture No. 3 the following daily growth of each pair of roots was determined: 5 and 5.5 mm.; 3.5 and 0 mm.; 1 and 5 mm.; 1 and 5 mm.; 2 and 5 mm.; 2.5 and 3.5 mm.; 0 and 3 mm.; and 2 and 5 mm. The growth during the first day in the gas of the roots of No. 4 was 10 and 5 mm., but for 5 days after that the growth was not only little but extremely irregular. On the sixth day it was 4 and 0 mm. and nothing the day following. In this culture it was noted that during a portion of the time the tip of one of the roots had turned away from the wall of the tube, although the other root-tip remained in position. In No. 5 the growth was equally irregular, as, for example, during the first day in the mixture the two roots increased 5 and 1.5 mm. and the last day the measurements were 0 and 4 mm. On the day next preceding the last the growth of each was 7 mm., and for 3 days in the midst of the test a growth of only 4 mm. took place.

In the second series of experiments with the barley, cylinder carbon dioxid only was used, and its affect on root-growth of four cultures was observed. Growth of the roots of each culture was demonstrated during a 24-hour period immediately preceding the gas administration. The temperature of the soil was 23° C. The gas was given 48 hours continuously, during which time no growth of the roots took place. It should be noted that in the cylinder carbon dioxid used there was 3 per cent or less of oxygen present, so that the observed effect is probably attributable to the carbon dioxid rather than to the lack of oxygen.

The experiments with barley seedlings appear to indicate that the species may be relatively insensible to poor soil aeration, particularly to a deficiency of oxygen, but that carbon dioxid in large excess is inimical to root-growth. The experiments, however, with carbon dioxid were not sufficiently extended to warrant more extended generalizations in this regard.

During the course of the experiments with barley some tests were made to learn whether an excess of carbon dioxid, or a deficiency of oxygen, would directly affect the rate of the growth of the shoot as well as of the root. In these tests auxographs of the type shown in figure 4 were used, which gave a continuous record of the growth of the shoot. The leading results can be referred to in this place. They were, in brief, not consistent. In some instances the growth of the shoot stopped at once upon administering the gas to the root, but in other instances this seemed to have no effect. Apparently shoot-growth ceased, where this took place, sooner with the roots in an atmosphere of carbon dioxid than when nitrogen was

employed. It should be stated that the experiments on shoot-growth were carried out in a room where the temperature was practically constant throughout the 24 hours, and where the humidity was fairly high and was constant as well.

Juncus effusus var. brunneus.

The species of *Juncus* studied is indigenous to the region about Carmel, where it occurs in low situations, where the soil is saturated with water much if not all of the year.

In the aeration experiments with *Juncus*, atmospheres both deficient in oxygen and rich in carbon dioxid were employed. The mixtures consisted of 2.1 per cent oxygen, with nitrogen, and oxygen-free nitrogen, in one series, and 10 and 48 per cent carbon dioxid with atmospheric air in the other series.

Transplanted mature plants were used. The manipulation was not without some difficulty, from the fact that the leaf-bases are crowded in such manner as to make sealing a difficult matter. At the beginning of the studies the seal was placed about the bases of the leaves, for which purpose it was necessary to use melted paraffine and beeswax in proper proportions. Even with such precaution slow leakage occasionally took place. In the end, therefore, the seal was placed below the leaves and around the root-crown, in which position no gas leakage occurred. The manner of sealing, and the reasons for this, was, in fact, about as in the case of the onion, already mentioned.

In the first series, on the relation to oxygen, 5 cultures were used. While in normal soil-air it was found that the rate of root-growth, at the temperatures employed in the experiments, was from 7 to 20 mm. daily. The first experiment with an oxygen deficiency was with 2.1 per cent oxygen and lasted for 9 consecutive days. During this time continuous connection was maintained with the gasometer and the gas was under slight pressure.

The cultures were fairly uniform in their reaction to the gaseous mixture, so that it will not be necessary to give a history of more than one of them. That of No. 1 is typical of the rest. The root of this plant increased in length, during 12 hours just preceding the administration of the gas, 4.5 mm. The soil-air of the tubes was then replaced by the gaseous mixture and, as before mentioned, permanent connection was kept with the gasometer, and the gas was under slight pressure. It therefore was supposed to be static. However, for a portion of the time, the seals leaked slightly, allowing a slow change of the experimental soil-atmosphere. The days on which this occurred are marked with an asterisk (*) in the measurements of the daily growth given below. The first 12 hours in the 2.1 per cent oxygen the root of No. 1 grew 3 mm., or at a rate nearly as great as in normal soil-air. The subsequent growth in 24-hour periods was as follows: 7*, 2, 10*, 4, 1, and 3 mm.

Culture No. 1 was given cylinder nitrogen, after it had been passed through wash-bottles containing alkaline pyrogallol, to learn whether root-growth would continue in the absence of oxygen, or in the small amount which would remain when so treated. The gas was used 24 hours, during which period no growth of the root occurred.

At the same time No. 1 was given the oxygen-free (?) nitrogen, and the same gas was given in Nos. 2 and 3 and at the same temperature. The results, in this case, did not harmonize with those obtained with No. 1, inasmuch as a root-growth of 2 and 3 mm. was seen to have taken place.

In order to obtain, if possible, more consistent results, another experiment was started in which the cylinder nitrogen was more completely

deprived of the small amount of oxygen it contained by being passed very slowly through alkaline solutions of pyrogallol. Two fresh cultures not previously used were employed. The reaction of the two was essentially the same, so that the history of one only need be given. The root of this culture was given the oxygen-free (?) nitrogen for 6 consecutive days, during which time the growth for 24-hour periods was as follows: 7*, 4.5, 6.5, 5.5, 5, and 3.5 mm. On the first day there was a very slow leak outward, which was promptly stopped.

Although it is apparent from the last experiment that *Juncus* has an extremely small oxygen requirement, it does not necessarily follow that root-growth in the species will continue for any appreciable time in the entire absence of that gas. It is not improbable that had the gas been passed through a reducing furnace, and had other suitable precautions been taken during the course of the experiment whereby all of the oxygen was removed and none entered the culture for any reason, growth of the root would probably not have occurred; at least such has been the experience with other species, which occur in analogous habitats, and which react in a similar way to cylinder nitrogen which had been treated with alkaline pyrogallol in the manner above described.

A single series was run in which the gaseous mixture used contained 48 per cent carbon dioxid with the balance atmospheric air. In this mixture, therefore, the amount of oxygen present was far in excess of the minimum for root-growth in *Juncus* as determined in the earlier experiments.

Three cultures were used in the second series, in one of which two roots were in view. During a 24-hour period just preceding the administration of the gaseous mixture the growth of the root was as follows: 3, 4.5, and 4.5 (two roots), and 2.5 mm. The mixture was then administered 48 hours continuously at a soil temperature of 22.5° C. As a result it was seen that in two of the cultures root-growth ceased promptly and was not resumed during the experiment. In the third culture, however, a growth of 2.5 mm. was recorded for the first day and of 1 mm. for the second day in the mixture. It was thought that in the last-named culture a slow leak may have developed.

The first experiment in the second series was with 10 per cent carbon dioxid. The experiment lasted for 8 days, during which period the gaseous mixture was given 7 days continuously. In normal soil-air, and for 24 hours before the gas administration, a root-growth of 2.5 mm. was recorded. The subsequent history of the culture is as follows: During the first 24-hour period in the gas, when two roots of the culture were observed, the growth was 7.5 and 3 mm.; for the second day it was 4 and 2 mm. After this time the growth of the second root only was measured, and this amounted to 10 mm. for the succeeding 5 days.

The results of the use of an excess of carbon dioxid appear to indicate that although the roots are not affected by a gaseous mixture containing 10 per cent, they are by one with 48 per cent of that gas. In absence of further results it appears, therefore, that even if the roots of *Juncus* may be tolerant of such partial pressure of carbon dioxid as is likely to obtain in their habitat, they, however, as compared to other species, are relatively intolerant to its presence in excessive amount.

Lathyrus odoratus.

Two experiments were carried out on the sweet pea in which a soil-atmosphere deficient in oxygen was used. In one the diluting gas was nitrogen and in the other it was helium. The seedlings were past the

"grand period" of growth of the main root, and laterals were present when the tests were made. No especial attention was given the temperature, although it was the same for each experiment and quite favorable for root-growth in the species.

In the experiment in which nitrogen was used, cylinder nitrogen, with 0.5 per cent oxygen, was given 48 hours continuously. At the outset, and while the roots were in normal soil-atmosphere, growth of the roots was demonstrated, but as soon as the gaseous mixture, deficient in oxygen, was given, growth of the roots ceased. Upon aeration, growth was resumed. In the specimens used, however, it was noted that although root-growth ceased in the mixture and was resumed upon aeration, the main root did not grow again, but only the rootlets. There a well-marked differentiation might be seen in the effect of the gaseous mixture used, even if it was not to be discerned during the gas administration itself.

The results were various in the experiments with helium, in which, as noted elsewhere, the percentage of oxygen actually employed was slightly below that when nitrogen was the diluting gas. In the static experimental atmosphere root-growth ceased, but when it was caused to stream slowly through the cultures growth continued at an apparently normal rate for 72 hours.

Lycopersicum esculentum.

In experiments on seedlings of the garden tomato, only the reaction to a deficiency of oxygen was observed. No especial attention was given the temperature of the soil, although it was known to be constant and suitable for growth in the species.

Cylinder helium was used. This, as shown elsewhere in this study, carried 0.2 per cent oxygen, which is somewhat less than the percentage in cylinder nitrogen. Root-growth was demonstrated before the gas was given. The administration lasted 48 hours and the gas was given statically. No root-growth took place during this time.

It should be pointed out that although it is evident much remains to be done on the aeration relations of seedling tomato, nevertheless the species and the variety used can not be said to be especially resistant to oxygen deprivation. With the percentage of oxygen employed, and with helium, root-growth in such species as the willow, for example, would be expected to continue, at least at a slow rate; the same is true of certain other species. What the critical percentage of oxygen for tomato may be, however, naturally does not appear from the study.

Medicago sativa.

A few tests were made on the reaction of the main root of alfalfa seedlings to a soil atmosphere poor in oxygen. In one set cylinder nitrogen, which carries 0.5 per cent oxygen, was used, and in another set 2 per cent oxygen, with helium, was employed. The experiments were continued for 2 weeks. The roots were maintained at constant temperature, but the shoots were subject to such temperature changes as took place in the laboratory. Especial care was taken to insure an adequate supply of water. The soil was constantly moist, but was not saturated, or puddled, at any time.

The first experiment was with 2 per cent oxygen. Immediately preceding the gas administration a root-growth of 11 mm. was noted in 48 hours. The cultures were then given the gaseous mixture for 24 hours, at the same temperature as before, but they were under observation for some time longer. In the experimental atmosphere, and for one day, the growth of the main root was 7 mm. Growth then ceased and was not renewed during the balance of the experiment.

In the second series of experiments, when 0.5 per cent oxygen was used, the results were not consistent. The first test lasted 48 hours, during all of which time the roots were in the gaseous mixture. The total growth was 5 mm. In order to test the validity of the results, fresh specimens were added and the same mixture given for 96 hours. In one of the plants no root-growth took place, but in another the growth was 3 mm. the first day, 4 mm. the second day, and none thereafter.

In an endeavor to obtain consistent results, further experiments were planned with 0.5 per cent oxygen. The tests lasted several days. Four fresh specimens were added to those already used. The growth was measured each day. As a result, it was found that while root-growth took place in all of the plants the first 24 hours, only one specimen showed growth subsequently.

Although not wholly satisfactory, the results appear to indicate that alfalfa is not especially tolerant of a small supply of oxygen. This is rather unlooked for in face of the fact that the roots of alfalfa, at least in cultivation, may penetrate the ground very deeply. At the time the experiments on the species were carried out, it was not known that temperature of the soil was as important a factor in root-growth, in case of oxygen deficiency, as was subsequently proven to be the case. The possibility remains, therefore, that had a soil temperature been used similar to which the roots would be subject under conditions of cultivation, root-growth would have gone on even in cylinder nitrogen. This is purely hypothetical, it should be remarked, although possible. But the possibility is not precluded that the species is, in fact, no more tolerant of a deficiency of oxygen than certain others of the same family, as, for example, the Canadian field pea. The experiment should be repeated, therefore, with especial consideration of the temperature relations of the species, including the cardinal temperatures for growth.

Mesembryanthemum æquilaterale.

The species of *Mesembryanthemum* used occurs naturally at Carmel and vicinity, as well as elsewhere on the coast of California, on dunes near the sea. It is frequently utilized as an ornamental, and as such is transplanted to localities more or less remote from the proper habitat. Thus, at Carmel, it is fairly common about town, where it may occur to the total exclusion of other species. But, wherever locally found, it is confined to sandy soil, which, accordingly, is well aerated at all seasons and is warm.

The experiments with *Mesembryanthemum* were carried on partly outdoor, under the oak trees at the Coastal Laboratory, and partly in the greenhouse laboratory. In the former no attempt was made to control the temperature, but otherwise the experiments were carried on as inside, except for the fact that rubber balloons were used as gas containers in place of the metal or glass gasometers more commonly employed. The temperature of the air ran from about 11.7° at night to about 20° C. by day, at times of clear weather. But when there was fog it varied little from 18° C. at any time.

The experimental mixtures used were either poor in oxygen or contained large percentages of carbon dioxid. Thus, in addition to observing growth in normal soil-air, studies were carried on with 0.5 per cent oxygen and 2 per cent oxygen with nitrogen, and with 75, 48, 45, 42, and 26 per cent carbon dioxid with the balance atmospheric air.

At the beginning of the outdoor experiments it was noted that the roots increased daily in length from 6 to 19 mm. in normal soil atmosphere, and at uncontrolled air-temperatures. The first series of experiments had to

do with root-growth in a diminished supply of oxygen in the soil. In these experiments 2 per cent oxygen was given 2 cultures continuously for 4 days. The temperature averaged relatively low. In culture No. 1 a root-growth of 2 mm. took place during the first 48 hours in the gas and 4 mm. the third day. Growth occurred also on the fourth day, but the root turned away from the tube-wall and the tip was lost to view, a not uncommon occurrence in a small supply of oxygen. Root-growth was questionable in culture No. 2 the first 24 hours it was in the gaseous mixture, but on the 3 succeeding days it was 3, 3.5, and 2 mm. for each day.

In order to have possibly more favorable conditions of soil-temperature for root-growth, as well as to have it under control, the two cultures were removed to the greenhouse and placed in soil thermostats which were running at about 21° C. They were under observation for 2 additional days under such conditions. The root of culture No. 1 increased 8 and 9 mm. on each of the 24-hour periods, while the daily growth in No. 2 was 5 and 6 mm. At the end of this experiment, therefore, the roots had been given a soil-atmosphere containing 2 per cent oxygen for 5 days and showed capacity for a fairly active rate of growth at the end of that time.

Immediately following the experiment last described, the two cultures were given 0.5 per cent oxygen for 7 consecutive days, and in the case of No. 1 for 10 days. The atmosphere used was static, but on the fourth day fresh gas was passed through both cultures. As a result it can be said in brief that growth took place each day in culture No. 1 for 7 days, although the amount was small. On the first days, however, and doubtless because of the freshly used mixture, the growth of the roots was the greatest of any 24-hour period. The actual root-growth of No. 1 for each day of the second experiment was as follows: 9, 6, 1, 2.5, 5, and 3 mm. On the seventh day the root-tip was hidden by soil, but it appeared on the eighth, when the growth for the 2 days was seen to be 8 mm. No additional growth of the observed root occurred during the 3 closing days of the experiment. In culture No. 2 a growth of 5 and 6 mm. occurred the first two 24-hour periods and an unmeasured amount subsequently. In the latter instance the root-tip curved away from the wall of the tube and did not reappear.

Reaction to an excess of carbon dioxid was taken up in the second series of experiments with *Mesembryanthemum*. The composition of the gaseous mixtures was such that the amount of oxygen in each was sufficient for a certain growth-rate, as determined by the experiments above presented. The observed effects, therefore, should be ascribed to the use of carbon dioxid.

In the first experiment, which lasted 13 days, 26.6 per cent carbon dioxid was employed. During a 24-hour period preceding the gas administration the root of No. 1 increased 5.5 mm. and that of No. 2 increased 9.5 mm. while yet in normal soil-air. The root-growth of the same cultures the first 24 hours in the gaseous mixture was 2 and 3 mm. for each, respectively, and for the second 24 hours 0.5 and 1 mm. Constant connection with the gasometer was maintained for 10 days longer, and it was observed that although the main root almost, or quite, stopped growth, there was a well-marked development of laterals.

A single set of experiments with 3 cultures and extending over 11 days was run, at a soil temperature of 25.5° C., in which the mixture contained 45 per cent carbon dioxid. During 24 hours immediately preceding the beginning of the experiment, and while the roots were in normal soil-air, a growth of from 5 to 5.5 mm. was recorded. In this experiment the normal

soil-air was replaced by the experimental mixture, after which the cultures were disconnected from the gas for a period of 48 hours. The roots behaved variously, although a certain amount of growth occurred in each root. In general, the growth on the second day was somewhat more than on the first day, but in No. 3 it was about the same. In No. 1 the growth of the root was 0.5 and 1 mm. for the 2 days, and in No. 2 it was 6.5 and 7 mm.; but in No. 3 the daily growth was about 13 mm., or quite as much as would be expected under normal conditions of soil aeration.

After having been left as above described for 4 additional days, the gas was again run through the cultures and was put under a small pressure so that any leakage which might occur would be outward. This seemed to be necessary following the inconsistent results as above described. In this condition the cultures were left for a period of 6 days, at the end of which it was found that a total growth of 18.5, 19, and 30 mm. had been made by the roots of Nos. 1 to 3, respectively. Finally, all of the cultures were aerated and left with normal soil-air for 24 hours, when the growth was from 3 to 6 mm.

A single experiment was run with two specimens of *Mesembryanthemum* in which 48 per cent carbon dioxid was used. In this experiment the results were wholly negative, although the roots appeared to be entirely normal when it was stopped. The reason for the growth cessation, in view of the earlier experiences, was not apparent.

In order to observe the immediate effect of an excess of carbon dioxid in the atmosphere of the soil an experiment was rigged in which the root-growth was determined by means of a horizontal microscope. The mixture contained 75 per cent carbon dioxid with atmospheric air. The temperature of the laboratory at the time was 27° C. As the leading result it can be said that during the first 45 minutes the root increased 0.2 mm. in length, and during the following 30 minutes the increase was 0.1 mm.; but in the third period, also 30 minutes, it shortened by 0.2 mm., and at the end of 8 hours it was found that a net gain of 0.6 mm. had been made. Upon repeating the experiment, substantially the same results were obtained. The interesting fact is apparent, therefore, that a certain amount of growth of the roots of *Mesembryanthemum*, for a short time at least, can go on in 75 per cent carbon dioxid.

Another experiment was set up in which the horizontal microscope was also used, but in which the gaseous mixture contained only 25 per cent carbon dioxid, with the balance atmospheric air. The experiment lasted 7 hours, during which time frequent observations were made, and at the end of that period it was found that the increase in length had been 6.5 mm.

Thus it appears that *Mesembryanthemum*, although occurring in sandy soil, can, nevertheless, endure surprisingly poor conditions of soil aeration. That is to say, the growth of the roots will continue not only in a great deficiency of oxygen, but also when the amount of carbon dioxid present is very large.

Nasturtium officinale.

A single series of experiments was run with water cress. Seeds obtained in the market were sown directly in tubes, so that transplanting was avoided, which, it should be said, was the procedure usually followed, but which is quite necessary in *Nasturtium*. The temperature of the cultures was kept at 23° C. The gaseous mixtures were cylinder helium and cylinder nitrogen, of which the former contained 0.2 per cent oxygen and the latter 0.5 per cent.

At the beginning of the test, root-growth of 2 cultures during 24 hours while in normal soil-air was seen to be 1 and 5 mm. This was immediately before gas administration. Then 0.5 per cent oxygen, with nitrogen, was given continuously 72 hours. Growth of the roots ceased as soon as the soil-air was replaced with the experimental mixture and was not renewed during the gas administration. The cultures were then aerated and left for 24 hours. Under such conditions of good oxygen-supply the roots increased 0.5 and 2 mm. in length.

In a second experiment, cylinder helium was given the cultures, also, but for 5 days continuously. In case of one, a slow rate of growth took place for 4 days, and in the second culture the root-tip turned sharply from the inner wall of the culture tube and was lost to view. In both cases, however, a certain amount of growth went on in the mixture used.

Although so limited observations do not permit important conclusions to be drawn, they do, however, suggest interesting possibilities, particularly when comparison is made with other species. As is well known, the water cress is confined to moist habitats, and occurs with especial abundance and is especially vigorous where the roots are in running water. Under such conditions the rate of delivery of oxygen to the roots is probably rapid, but is possibly less than in porous soil, such as sand. The suggestion, therefore, seems pertinent that the high degree of tolerance to a small supply of oxygen is related to the aeration conditions of the subterranean habitat commonly occupied by the roots of the species; and it is of interest to note, in this connection, that species which naturally occur where the soil may be puddled all or a part of the year exhibit greater tolerance to an oxygen deficiency. Such species is *Juncus*, the oxygen relations of which are referred to in another place.

Opuntia versicolor.

Field studies on *Opuntia* show that the roots of any individual are of two kinds, namely, those which go directly downward from the base of the shoot and serve the purpose of anchoring the plant, and those which spread laterally and appear to function mainly as absorbers of water. Thus root development in the species, and in the family for that matter, is highly characteristic, and the question arises as to whether the type is obligate or, if not, to what extent it may possibly be modified by changes in the environment. To investigate this problem two types of experiments were planned, of which one was with cultures in the garden and the other with those in the laboratory, where certain features of the soil environment might be measured and controlled.

In one type of garden culture cuttings were grown for 2 years in two kinds of soil, namely, sand and adobe, and in the other outdoor culture cuttings were grown in large and long tubes or pipes. The immediate purpose of the experiments was not the same. In the first it was proposed to watch the effect of poor aeration, as contrasted with good aeration, on root development, and the second was for the purpose of testing whether the roots of *Opuntia* could be forced in any way to penetrate the ground deeply.

The outdoor sand-adobe cultures were arranged as follows: A pit 1 by 2 meters in extent and 1 meter deep, was filled with sand from a wash on the domain of the Desert Laboratory. The sand was such as passed through a 20-mesh to the inch sieve. The experiment was carried out in the experimental garden of the Desert Laboratory. It should be said that the soil of the garden, which immediately surrounds the pit, is of adobe with an

admixture of fine stones. It is seen, therefore, that the physical characteristics of the soil of the pit and of the garden about it are quite different. Of these, it will be sufficient to remark that the former is not only better aerated at all times and in all seasons and has different moisture relations, but the temperature at any given depth may be quite different from that of the garden soil immediately adjacent. Often, however, the temperature of the pit was found to be relatively high. The soil in which *Opuntia* naturally occurs is not greatly unlike that of the garden where the pit was situated.

After the pit had been filled and was well wetted, large cuttings of more than one species of *Opuntia*, including the one now being discussed, were planted in rows crossing it. It therefore happened that some of the plants were wholly in adobe, others were wholly in sand, and yet others were placed on the line demarking the two.

At the end of two years the experiment was terminated. It was found that normal shoot-growth had taken place in all of the plants. The roots were then uncovered with care and the following leading features were found respecting the roots: The plants which were wholly in the adobe soil had a normal type of root development, that is, there were anchoring roots and absorbing roots, the latter of which reached out far beyond the shoot but lay very near the surface of the ground. The plants wholly in the sand had no proper superficial roots and no proper anchoring roots, but the roots extended downward at many angles and divided the soil-mass encompassed by them fairly equally. But the roots of the plants situated on the line separating sand and adobe were of both types. That is, those roots which were on the adobe side were either superficial and horizontal or went fairly directly downward, while those on the sand side developed after the manner of the roots wholly within the sand. Thus the important fact was demonstrated that the roots of *Opuntia* are exceedingly plastic and are directly affected by the immediate conditions of the soil environment.

In the second series of experiments cuttings were caused to send their roots downward through metal or glass pipes, or tubes. The tubes were kept outdoor and were subject to the temperature fluctuations of the garden air. The glass tubes used were 5 cm. or less in diameter and the galvanized-iron pipes had a diameter of 15 cm. The soil used was sand mixed with adobe. The cultures were occasionally irrigated, but were left otherwise undisturbed for about 24 months. The leading results, so far as they concern *Opuntia*, can be briefly given. Root penetration between 78 cm. and about 3 meters took place. There was little branching of the root in the middle of the tubes, but within 30 cm. or less of the ends the roots branched freely.

In considering the definitive reactions it was assumed that in the first series the roots were especially influenced by the temperature and the aeration of the soil and in the later series it is evident that during at least a portion of the time the temperature was appropriate for root-growth, but the conditions of aeration were not uniform throughout the tubes and might not be "good." Nevertheless growth occurred. As regards the moisture of the soil in the sand-adobe experiment, it did not appear to be the direct cause of root differentiation, for the reason that, during the growing-season, the soil was suitably wetted in the adobe, as in the sand, to a depth much beyond that occupied by the roots. Dryness of the soil, therefore, was not a hindering cause of root penetration. Accordingly, experiments were begun on the temperature and the aeration relations.

Temperature Relations of Opuntia.

As has appeared, both root and shoot growth occurred in the species when growing in the outdoor cultures at the Desert Laboratory garden. When, however, the same species was transferred to the garden of the Coastal Laboratory, it was found that neither the shoot nor the root grew, although the plants remained living for several years. This was thought to be attributable to the low soil-temperatures in the garden and to the further fact that the warm season at Carmel is without rain. When, however, the plants were irrigated, and they yet remained dormant, the condition observed was plainly owing to low soil-temperatures. To test this further an experiment was planned as follows: A galvanized sheet-iron box, 130 by 30 cm. and 90 cm. deep, was constructed in such manner that there were three compartments. Of these, the largest was centrally situated and was provided with a sloping glass with a removable shield to prevent the admission of light, as well as to prevent rapid radiation of heat from the soil. Carbon-filament incandescent lamps of rather small capacity were placed in the end compartments and were kept running continuously throughout the experiment. The exposed surface of soil, which was of sand and garden loam, was covered with heavy paper. The apparatus was left fully exposed in the garden of the Coastal Laboratory. In early autumn several "joints" of *Opuntia versicolor* were set next to the glass side of the middle compartment and were left without being disturbed in any way for a period of about 8 weeks. In this arrangement it will be seen that the shoots of the plants were subject to the varying temperature changes of the garden, and at times to relatively low temperatures, although there was no frost. Such of the roots, on the other hand, as were at the ends of the compartment were warmed by the heaters, but such as were in the middle portion were not warmed. The leading results of the garden experiment were, in brief, as follows: At the end of the 8-weeks period it was found that the shoots and roots of the plants in the middle or unheated portion of the compartment had not grown at all. But, on the other hand, the plants which were at the ends, where the temperature of the soil was about 30° C., had formed fresh shoots, bearing leaves as much as 10 cm. in length, and that much growth of the roots had also taken place. These results are particularly striking in view of the fact, as stated above, that the same species situated in the same garden during observation covering several years, and including the warm seasons, have not shown growth either of root or of shoot. That a fairly high soil-temperature is necessary for root-growth in *Opuntia versicolor* seems, therefore, to be clearly shown by this experiment. In the experiment, as will be noted, the aeration conditions were probably good for the reason that the soil used was coarse, being sand from the garden.

Uncontrolled experiments, in which the thermometer in the soil was read at 6^h 30^m and 6^h, covering a relatively long period, indicated that root-growth in *Opuntia* was dependent, among other factors, on relatively high soil-temperatures. Thus, during a period of 6 weeks in April and May, when the temperature of the soil was 26° C., or over, a total root-growth of 341 mm. occurred in one main root; but in November and December, during a period of 24 days, the total root-growth was only 89 mm. At this time the temperature of the soil was between 12.3° and 21.3° C., which is the average of readings made in early morning and late afternoon. The maximum daily root-growth of spring and of late autumn was 20 and 5 mm. respectively, and the minimum 2 and 1 mm. When the temperature changes were compared with the rate of growth of the roots it was found that in a general way the rate doubled with a rise of 10° C.

For periods up to 9 hours, when the temperature of the soil is kept constant, the rate of root-growth may vary from hour to hour to a small extent, but there does not appear to be a consistant decrease. The temperature at which the rate was observed to be most rapid for so long a period was 32.5° C. Such rate is about 0.8 mm. per hour. At a soil temperature of 34° C., the increase per hour has been seen to be 1 mm., although possibly for a shorter period. At 20° C., root-growth of *Opuntia* is about 0.6 mm., more or less, per hour, and at 16° C. it falls to about 0.07 mm. The temperature range for an effective rate of root-growth in *Opuntia* is, therefore, not large, and the minimum temperature for such growth is relatively high.

Studies on the temperature and moisture of the soil at the depth attained by the roots of *Opuntia* indicate that in southern Arizona there are only a few weeks in summer which are favorable for root-growth in the species.[1] Extending these conclusions to other species of cacti, it has been suggested that the family as a group is in the main restricted to warm temperate regions with summer rains, and that where introduced to regions where such conditions obtain they may be expected to survive. Where other conditions obtain, such as rainless summers, they are largely wanting. Nor do they occur where the appropriately moist soil is impossibly cool. The leading limiting factors in such general distribution, as well as in the physiological root activities of the local species, are apparently, therefore, moisture of the soil and low soil-temperature.

Aeration Relations of Opuntia.

In attempting to relate such findings to what is known regarding the differentiation of the root system of *Opuntia* into anchoring and absorbing roots, it is evident that the reactions have to do not only with the moisture and temperature of the soil, but with other factors as well, of which it is necessary only to mention possible variation in the aeration of the soil as between soils of unlike physical nature and unlike depths. Means were not at hand for determining the carbon dioxid-oxygen content of different soils at different depths and at different seasons, but experiments were organized by which the roots could be exposed to soils in which the percentages of these gases was known. The nature of the apparatus has already been described; a running account of the leading results follows.

It has been the practice to occasionally include two species of unlike root habit in the same experiment for the purpose of comparing the root reactions to the same conditions of soil aeration. To a certain extent this was done in the studies on *Opuntia* when the reaction of the root of *Prosopis velutina* was compared to that of *Opuntia*, both being at the same temperature and subjected to the same type of soil-atmosphere. In such studies cuttings of *Opuntia* and seedlings of *Prosopis* were used. Inasmuch as the temperature and aeration relations of *Prosopis* will be treated in another paragraph, only such features as were brought out in connection with the *Opuntia* studies will be presented in this place.

The experiments on *Opuntia* have been many and have covered several seasons. The leading results, however, can be given in a few paragraphs. In the tests high percentages of carbon dioxid and low percentages of oxygen have been used. Atmospheric air was used in part to dilute the carbon dioxid and nitrogen to dilute the oxygen. The experiments were partly short-time and with the horizontal microscope, but many of them were

[1] Carnegie Inst. Wash. Year Book No. 14, p. 87, 1915.

carried over relatively long periods, when the growth of the roots was read for the most part daily.

In a preliminary experiment with a deficiency of oxygen 0.8 per cent was used, with nitrogen, and *Prosopis* and *Opuntia* were observed for 8 days. The temperature was constant during the experiment and the two species were in the same thermostat. The gas was under slight pressure and continuous connection was made with the gasometer. As the leading result it can be stated that while the root of *Prosopis* increased 10 mm., in length the *Opuntia* root did not grow at all.

Experiments were set up in which cylinder nitrogen was caused to pass through wash-bottles containing alkaline pyrogallol and the customary precautions were taken to exclude oxygen from entering the culture through the seal. Whether all of the oxygen was taken out and kept out of the cultures in this way may be questioned, but the results are such as to make this of no importance. Experiments with *Opuntia* under such anaerobic conditions gave only negative results: therefore it appeared fairly well established that a certain amount of oxygen in the atmosphere of the soil is necessary for growth of the roots of *Opuntia*.

Nitrogen with 1 per cent oxygen was given the roots of an *Opuntia* cutting for several days. Growth occurred during the first 24 hours in the gas, but none afterward.

In experiments with 2 per cent oxygen, which continued for 11 days, root-growth was noted for 10 days, although the rate was relatively slow. The actual daily increments were 4, 5, 6, 4, 4, 3, 3, 4, 4, 2.5, and 0 mm.

An experiment was run in which 2.4 per cent oxygen was given several days continuously. Thus, during 5 days a root of one culture grew 13.5 and that of another 17 mm., while at the same temperature the root of the control increased 70 mm. in length.

In a soil containing 2.67 per cent oxygen the results were somewhat different from those above sketched. In this mixture root-growth was not only relatively slow, but after a period of 48 hours in the gas the root ceased to grow at all.

Numerous experiments were carried on in which the atmosphere of the soil contained 4.4 per cent oxygen with nitrogen. In one experiment, lasting 5 days, in which 2 specimens were used, root-growth was seen to go on throughout the entire period. In the two plants the entire root-growth was 23 mm. in one and 28 mm. in the other, which, however, is considerably below the expected average for the temperature used. For example, one root of the control in normal soil-air increased 47 mm. in length during the experiment.

Additional experiments with a relatively good oxygen-supply, as, for example, 2.5 to 4.4 per cent, showed that different plants may react in different ways. Thus, in one instance, root-growth ceased soon in 4.4 per cent oxygen, while in another culture it continued for over 10 days in 2.5 per cent oxygen.

The results in 7 per cent oxygen were also somewhat inconsistent. Thus, while growth might usually be expected in one instance, it stopped after the first 24 hours in a mixture containing so large a percentage.

In view of the inconsistent results with 4.4 and 7 per cent oxygen, an experimental atmosphere was made up with 10 per cent oxygen and was given for over 5 days. As a result, it was observed that although root-growth continued the whole time, it was at an ever-decreasing rate. In one series with 10 per cent oxygen observations were made on several roots of varying length, of the same culture. It was found that in one

instance 3 roots stopped growth very soon after the gas was administered, 2 roots continued to grow 1 day, 1 grew 3 days, and 1 grew 4 days. It chanced in this case that the roots which ceased earliest to grow were the shortest, and, conversely, that the one which grew the longest time was the longest of the plant. Analogous reactions were observed in other experiments on *Opuntia*, indicating, as will be seen, a differentiation in reaction between roots some of which might, under natural conditions, become anchoring roots and others absorbing roots. In such case the former would penetrate the ground fairly deeply, while the latter would remain near the surface. Other experiments with *Opuntia* confirmed the result of the one mentioned so far as regards the differential reaction between longer and shorter roots of the same plant to a soil atmosphere containing a subnormal, but, nevertheless, fairly large amount of oxygen.

In observing the effect of an excess of carbon dioxid in the soil on the rate of root-growth in *Opuntia*, the gas was used both with an adequate percentage of oxygen for growth and with a deficiency of oxygen. The experiments were numerous and were carried out both in short periods, when a horizontal microscope was used and without temperature control, and in thermostats, in which event the periods of observation were relatively long.

In experiments with 50 per cent carbon dioxid with atmospheric air, and at a soil temperature of 28° to 29° C., the roots were exposed to the mixture for a period of 3 hours. As a result it was found that although the root had increased 0.34 mm. in length during the hour preceding the gas administration, it grew only 0.2 mm. in the 3-hour test. At the end of the administration period the culture was well aerated, but about 2 hours elapsed after the gaseous mixture had been replaced by air before the normal rate of root-growth took place.

Higher percentages of carbon dioxid with atmospheric air were used at temperatures which were in part near the optimum for root-growth in *Opuntia* and in part not far above the minimum for growth. The periods were short, as before. The leading results can be summarized in a few sentences. The gaseous mixture first used contained 75 per cent carbon dioxid. At a soil-temperature between 27° and 32° C., and during an exposition time of 3 hours, root-growth either decreased greatly in rate or wholly stopped. For example, in one experiment and for the period given, the total growth was 0.5 mm. in one root and 0.2 mm. in another. The cultures were then well aerated and it was found that at the end of 3 hours in normal soil-air the growth became 1.6 and 1.5 mm. for the same roots.

In other experiments with 75 per cent carbon dioxid the temperature of the soil was 16° C. At such low temperature and in so high percentage of carbon dioxid, for a period of 2 hours, root-growth ceased in one instance and was 0.2 mm. in another. Atmospheric air was then used to replace the excessive amount of carbon dioxid, and it was found that at 16° C. growth of the roots was not resumed in 3 hours, but when the temperature of the culture was raised to 29° C. a growth of 1.2 mm. took place in the following 3-hour period.

In subsequent experiments the effect of even higher percentages of carbon dioxid with atmospheric air was observed, and in several instances the relation to the temperature of the soil was noted. Thus, in a mixture containing 85 per cent, the growth of *Opuntia* roots ceased either immediately upon the beginning of the gas administration or very shortly afterward. In one experiment a growth of 0.2 mm. took place during an exposition of 3 hours, but in another experiment, soil temperature 26° C.,

growth ceased with the gas administration. So far as recovery from the effects of a gaseous mixture containing 85 per cent carbon dioxid is concerned, it was observed to take place sooner at higher than at lower soil-temperatures.

Experiments in which 95 per cent carbon dioxid was used gave results essentially the same as when the percentage was somewhat less. That is to say, cessation of root-growth, or its marked decrease in rate, followed a gas administration for 3 hours. There also was a relation between recovery from effect of the gas and the soil-temperature. In one experiment at optimal soil-temperature a root-growth of 0.2 to 0.5 mm. took place during 3 hours, and recovery occurred in about 3 hours after the culture was aerated. At soil temperatures from 3° to 7° C. below this, however, the effect of the mixture was more pronounced during the exposure, and the recovery in normal soil-air subsequent to it was not so soon as at the higher temperature. When the temperature was increased to 35° C., which is above the optimum for root-growth in *Opuntia*, the depressing effect of the gas was even more striking and the recovery was comparatively slow. Thus, root-growth measurements of 3-hour periods during and after the administration of the gaseous mixture containing so great excess of carbon dioxid were 1.2 and 0.1 mm. in the administration period and 0.6 and 0.9 mm. in normal soil-air.

Further experiments, of short duration, were run with the cylinder gas. The length of the periods of gas administration varied from one-half hour to 3 hours, and both optimal and suboptimal soil temperatures were employed.

In experiments carried out at a soil temperature of 19.9° C., a growth of 1 mm. took place in 1 hour, of which the gas was administered 30 minutes. So great growth, however, in carbon dioxid was not had in other experiments. Of these it need only be said that at 22° and 32° C. cylinder carbon dioxid given for 30 minutes, during an observation period of 1 hour, caused a decrease in growth to 0.6 and 0.4 mm., for the two temperatures given.

In all of the experiments, recovery from the depressing effect occurred 1 hour or more after the cultures were aerated, but at the higher and optimal temperatures a fairly good rate, although below that characteristic for the temperature, was observed to take place during the first hour in normal soil-air.

The exposure of the roots of *Opuntia* to a soil atmosphere consisting of cylinder carbon dioxid, as before, for a period of 60 minutes showed depression in growth-rate, a quiescent period of greater or shorter duration following it, and usually, or always, ultimate recovery. The length of time necessary before the normal rate of growth was resumed was 2 or more hours after the replacement of the gas by atmospheric air. In these experiments the temperature of the soil was 22.7° to 23.3° C. throughout. The course of a single representative experiment may be given. This lasted 5 hours, with readings at the end of each hourly period. The gas was given during the first hour only and was then replaced with atmospheric air. The hourly growth increments were as follows: 0.2 mm., administration period, 0.1, 0.25, 0.25, and 0.6 mm.

The effect on root-growth in *Opuntia* of exposure to cylinder carbon dioxid for a period of 3 hours and at different soil temperatures, was observed in experiments which are summarized in table 14. The gas was given during the first period, when it was replaced with atmospheric air. During the following periods, therefore, the roots were in normal soil-air. The experiments were carried out on successive days. The leading results

can be said to be, first, growth cessation or depression of the rate of growth during the gas administration, followed by fairly slow but ultimate recovery, and second, recovery soonest at the higher temperatures.

TABLE 14.—*Root-growth of Opuntia versicolor in the presence of cylinder carbon dioxid for periods of 3 hours, and on 3 successive days.*

(Gas was administered during first period.)

Plant No.	First period.	Second period.	Third period.	Fourth period.
First day, 22° to 23° C.:	*mm.*	*mm.*	*mm.*	*mm.*
[1]1	0.8	1	1.3	1.1
2	.3	1	.8	1
3	0	0	0	1.3
Second day, 33° to 35° C.:				
[1]1	2.2	2.4	2.1	1.9
2	0	.3	.3	.7
3	.2	.5	.2	1.6
Third day:				
[1]1	0.7	0.5	0.9	0.7
2	0	0	0	.2
3	.1	.1	.2	.4

[1] Control.

In the preceding experiments with excessive amounts of carbon dioxid, there was a deficiency of oxygen for the reason that atmospheric air was used as the dilutant. The resulting effects might, therefore, be held to be in part at least due to this fact. Accordingly, a few experiments were carried out with excessive amounts of carbon dioxid, but in which enough oxygen was used to bring the percentage up to that of atmospheric air, or even somewhat higher. The following mixtures were used: 75 per cent carbon dioxid and 25 per cent oxygen, equal parts carbon dioxid and oxygen, and 25 per cent carbon dioxid with 75 per cent oxygen. The temperature of the soil was in part low, about 20° C., and in part about optimal for root-growth.

The results were various. In the experiments in which there was 50 and 75 per cent oxygen, 50 and 25 per cent carbon dioxid, at low soil temperatures, the results were wholly negative. When 25 per cent oxygen was used, with 75 per cent carbon dioxid, however, the results were somewhat different. The temperature of the soil was 30° to 33.5° C. and the administration period 3 hours and 15 minutes. In these experiments it was observed that although no growth of the roots took place while they were in the mixture, as soon as the cultures were aerated growth was resumed.

The experiments clearly indicate that an amount of carbon dioxid in the soil equaling 25 per cent by volume of the soil-atmosphere exercises a specific effect. Although the immediate effect of an excess of carbon dioxid is to depress the rate of root-growth or to cause it to cease where the administration period does not exceed 3 hours, the growth of the roots is ultimately resumed when given atmospheric air. The length of the period of depression of growth-rate is apparently influenced both by the amount of carbon dioxid present and by the temperature of the soil. As

to the first, however, there does not seem to be a striking difference between the effects of a small and of a large excess of carbon dioxid, although such difference probably exists. Recovery appears always to be sooner at higher than at lower temperatures of the soil, although the reason for this was not learned.

Oryza sativa.

The cultivated rice is of especial interest in studies on the aeration relation of roots from the fact that in cultivation the plant is grown in water, more or less stagnant "paddies," and to the further fact, which is discussed in another part of this study, that it is the only known seed plant which will germinate in the proved absence of oxygen. The seed which was used in the aeration experiments was of Wateribune rice, from Briggs, California, and was furnished by the Bureau of Plant Industry, U. S. Department of Agriculture.

Two series of experiments were planned, one of which was to test the capacity of the species for root-growth under anaerobic conditions, and the other was to observe the direct effect on root-growth of an excess of carbon dioxid in the soil. Seedlings were used of which the roots were 22 cm. or less in length. The temperature of the cultures was various. In some instances it was 22.5°, in others 26° C., and in yet others it was intermediate between the two.

In the oxygen series, which contained 0.5 per cent oxygen, cylinder nitrogen was used exclusively. The experiment included 4 cultures and was run continuously for 2 weeks. In the conduct of the experiment irrigation was effected in two ways, of which one was by turning the tubes upside down, by which water ran from the reservoir into the soil, wetting it sufficiently, and the other was by opening the cultures and adding water from without. In the latter event some atmospheric air was introduced which had to be replaced each time by the gas. It therefore happened that a portion of the cultures received a certain amount of oxygen occasionally other than that provided for by the test itself. The effect of this is shown in the observations on the reaction of the roots.

The experiment was begun September 13, the temperature of the soil being 23° C. While in 0.5 per cent oxygen the first 24 hours, the roots of 3 of the cultures increased 1 mm. in length and that of the fourth 5 mm. Nos. 1 and 2 were irrigated by the open method and Nos. 3 and 4 by upending at the beginning of the second day and at the beginning of the third day of the experiment. For the preceding 48 hours the root-growth of the 4 cultures was 6.5, 8, 24, and 2 mm. The behavior of No. 3 indicated that the gas had passed slowly through the soil, by which means the roots thus obtained a small but sufficient supply of oxygen for an active rate of growth. From this day, September 16, until the morning of the 20th, the root of No. 1, with open irrigation, grew 20.5 mm., and No. 3, with closed irrigation, increased only 2.5 mm. in length. The subsequent history of the roots, with one exception, is extremely variable. Culture No. 2 was broken, but No. 1 was continued with open irrigation until the last of the month. During this time, except for 3 days, the daily increase in length of the root varied between 3 and 7 mm. The root of No. 4 with closed irrigation, between September 18 and 20, 72 hours, increased 20.5 mm. and after that growth was doubtfully noted. Root-growth in Nos. 3 and 4 was doubtful after the 20th.

The general course of the experiment has been given for the purpose of showing that the roots of rice seedlings can maintain a fairly active rate of root-growth in a very small amount of oxygen, but that in its absence

this is not probable. And although in this experiment analyses were not made of the gas of each culture, it is likely that in the closed cultures the small amount of oxygen present was consumed more rapidly than it was received by the roots, thus bringing about growth cessation. But, on the other hand, when the gaseous mixture was passing slowly through the soil, or when the cultures were opened upon being irrigated, the response on the part of the roots was prompt and unmistakable. The experiment, therefore, indicates that the roots of rice can not grow in the absence of oxygen, but that the amount required for growth is not large.

In the second series of experiments, the roots were given an excess of carbon dioxid with a varying amount of oxygen, but an amount which would be sufficient for root-growth. The reactions, therefore, were to do, and probably wholly so, with the high percentage of carbon dioxid present. The gaseous mixtures employed were 26.6, 48, and 49 per cent carbon dioxid with atmospheric air, and cylinder carbon dioxid which contained about 3 per cent oxygen. The temperature of the soil was between 22.5° and 26° C. Care was taken to see that the soil was continuously moist, although it did not become saturated with water at any time.

In the first experiment with carbon dioxid, 5 cultures were run, of which root-growth occurred or was demonstrable in 2, of which one had 2 roots in view. The growth of the roots, while in normal soil-air for 24 hours immediately before the gas administration, was 4.5 mm. in No. 2, and 4.5 and 7 mm. in No. 4. The gas, 26.6 per cent carbon dioxid, was given September 1, and on the following day it was observed that slight growth had taken place in No. 2, the tip of the root of which had turned away from the wall of the culture tube, and in No. 4 the growth of the two roots was 1 and 2.5 mm. The cultures were then opened and irrigated, and again given the gas for 24 hours. At the end of the second 24-hour period in the mixture, however, it was seen that no additional growth had taken place. The experiment was continued 24 hours longer, but growth was not resumed. Finally, all of the cultures were aerated and were observed for 96 hours longer, but no growth of the roots took place.

In a mixture containing 48 per cent carbon dioxid and at a soil-temperature of 25° C., an experiment was run with 2 cultures for 72 hours. A growth of 23 mm. took place while in normal soil-air in No. 2, in which 2 roots were in view, and which was 22 cm. in length at the beginning of the experiment. The gaseous mixture was then given for 24 hours, and it was found that during this time the root of No. 1 had grown 2.5 and the longer one of No. 2 had grown 6 mm. A root of No. 2, which was 17 cm. in length, did not grow in the excess of carbon dioxid. The root of No. 1 did not grow subsequently, and during the second day in the gas that of No. 2 grew only 1.5 mm. and none afterward.

On August 21 to 25 an experiment was run with 3 cultures of rice in which 49 per cent carbon dioxid, with atmospheric air, was used, or approximately the same mixture as that above given. The temperature of the soil, however, was 22.5° to 23° C. In the same thermostats and at the same time with rice there was carried a single culture of *Potentilla*, for comparative purposes.

At the beginning of the experiment, growth of the roots was observed for 24 hours in normal soil-air. In No. 1, for 8 days, it was 16.5 mm. In Nos. 2 and 3, for 24 hours, it was 9.5, and in No. 3, two roots, it was 11.5 and 14.5 mm. Finally, in *Potentilla* it was 5 mm. The gaseous mixture was given August 22, and on the following day it was found that slow outward leaks had developed in rice culture No. 1 and in the *Potentilla*

culture. The root-growth of all of the plants during the first 24 hours in the gas was as follows: In No. 1, none; in No. 2, 5 mm.; in the two roots of No. 3, 5 and 4 mm.; and none in *Potentilla*. It will therefore appear that growth of the roots took place only in the cultures which were gas-tight, and ceased in the others, in which there was a slow leak. This was corrected August 22. All cultures were irrigated on the 23d and were given the gaseous mixture for 24 hours more. On the 24th the following growth measurements were made for the preceding 24-hour period: 0.5 and 3 mm., two roots, in No. 1; 1 mm. in No. 2; 0 and 2.5 mm., two roots, in No. 3; and 0.5 mm. in *Potentilla*. The roots were left an additional 24 hours in the gas, when it was found that there was no growth in Nos. 1, 2, and in *Potentilla*, and in No. 3 one of the two roots had grown 2 mm. and the other not at all.

The results of this experiment, therefore, are somewhat conflicting. It would appear, however, that in the case of slowly streaming gas the results are to maintain the percentage at a strength equaling that in the reservoir, with consequent striking results on root-growth. It appears quite possible, on the other hand, that statically used the gas may diffuse out of the culture and very possibly atmospheric air may diffuse inward, as oxygen is known to do, thus effecting a marked alteration of the composition of the gaseous mixture. Only individual gas analyses of the cultures could determine this point, and these were not made.

In the final experiment, on the effect of carbon dioxid on root-growth in rice, the cylinder gas was used. The experiments were carried on for 6 days at a soil temperature of 26° C. Six cultures were used, in which 2 or 3 roots in each were under observation. The results were not so uniform as might be expected from the reaction of the roots to an excess of carbon dioxid as seen in the earlier experiments.

At the end of the first 24-hour period in the gas the following condition was noted: In No. 1 three roots had grown from 1 and 2 mm. in length. This culture was tight. The root of No. 2 showed essentially the same growth as No. 1 and it also was quite gas-tight. There was found to be outward gas leakage in Nos. 3 (very slow), 4, and 5, of which that of No. 4 was very considerable. In culture No. 3, two roots showed no growth, but the third increased 1.5 mm. in length. The two roots in No. 4 grew 15 and 16.5 mm., and those in No. 5, with a much slower outward gas leak, grew 2, 2.5, and 1.5 mm. The growth in No. 6, which was tight, was essentially as in No. 1. It appears, therefore, that cylinder carbon dioxid, given 24 hours, did not inhibit root-growth in the rice seedlings.

On the morning of the third day in the gas there was no growth in No. 3 and No. 6; in No. 2, one of the roots had grown 0.5 mm. and the other two none; in No. 5, two of the roots had grown 2.5 and 7 mm., and in No. 4 the root-growth was 18.5 and 17.5 mm. Two roots of No. 1, however, grew 4.5 and 6.5 mm.

On the next morning the following conditions were found: In No. 1 the growth was found to be 1 and 1 mm. for the preceding 24 hours, but none had taken place in Nos. 2, 3, and 6; in No. 4 the growth of two roots was 13.5 and 16.5 mm., and in No. 5 one root did not grow, while the other increased 4 mm. in length.

The experiment was continued for three 24-hour periods longer. At the end of the first period there was seen to have been no additional growth in Nos. 1, 2, 3, and 5, and doubtfully in No. 6, but the roots of No. 4 had grown 5.5 and 7.5 mm. In this instance all of the tubes, including No. 4, were gas-tight.

During the next 24-hour period there was no root-growth in Nos. 1, 2, or 3. In the case of No. 4, which hitherto had grown at a surprising rate, the roots had become about 1.5 mm. shorter, although otherwise of normal appearance.

The gas was used streaming slowly the last period of 24 hours, and it was found that there was slight root-growth in Nos. 1, 5, and 3; 1.5 and 1.5 mm. in No. 2; and 1.5 and 2.5 mm. in No. 4.

Whatever the reasons may be for the inconsistencies of the results, it should be noted that, as a rule, the roots in any one culture reacted in general in the same way. The very good rate of growth of No. 4, during 72 hours of the 6 days, as opposed to the very slow rate or total cessation of growth in most of the other cultures, as well as the sudden and brief acceleration of growth of No. 1 the second day in the cylinder carbon dioxid, are difficult to understand. Owing to the fact that all cultures received gas from the same reservoir and under slight pressure, the same in all cases, there was only outward molar movement, when indeed such took place. Analogous results were obtained in the use of 48 to 49 per cent carbon dioxid, but only growth cessation resulted from using 26.6 per cent of the gas. This is curious, in view of the fact that the experiments with 26.6 and 49 per cent carbon dioxid and with cylinder carbon dioxid were carried on at about the same temperature. The results, however, suggest that the roots of seedling rice are capable of maintaining growth in extremely high percentages of carbon dioxid, and this, coupled with the further fact that growth will go on, although slowly, in so little as 0.5 per cent oxygen, indicates that the species is well adjusted to withstand conditions of poor aeration.

Phalaris sp.

A single experiment was carried out on seedlings of *Phalaris* which were from seeds purchased in the market. Cylinder nitrogen, with 0.5 per cent oxygen, was used. Four cultures were observed. The experiment was continued for 6 days.

At the beginning, and just before the gas administration, a root-growth of from 17 to 31 mm. was recorded for 24 hours. The gas was then given 96 hours continuously, with the following leading results: During the first period of 24 hours a growth of from 2 to 5 mm. took place in each culture. In 3 cultures no additional root-growth occurred, but in the fourth there was some growth the second day, but none thereafter.

Although no especial importance can be attached to the meager results, they appear to point to the possibility that *Phalaris* is rather more sensitive to oxygen deprivation than other grasses.

Phaseolus sp.

A few experiments on the aeration relations of the roots of a garden variety of bean and on the Papago bean of the Southwest were carried out, in which the direct effect on root-growth of an oxygen deficiency was observed. The experimental mixture consisted of from 0.5 to 10 per cent oxygen with nitrogen. Only "growing" soil-temperatures were used, but the cultures were kept in thermostats, so that the temperature throughout any experiment was constant and that of one experiment was like that of another.

In tests with the garden bean, 0.5 per cent oxygen was used. The experiment was continued 4 days, during which time the gas was given

continuously. In two cultures root-growth went on at a fairly slow rate during the first 24 hours of the experiment, 2.5 mm. in one and 8 mm. in another specimen, but dropped to 1 mm. during the second 24 hours, and then ceased entirely. When, however, No. 3 was aerated and irrigated, growth was resumed. In the last culture, also, it was noted that shoot-growth continued while the root was in the 0.5 per cent oxygen.

The tests with the Papago bean were made with 0.5, 7, and 10 per cent oxygen with nitrogen, and the gas-administration periods were from 24 to 48 hours. The seedlings were relatively old and had well-developed roots with laterals already forming. The leading results can be briefly given. In 0.5 per cent oxygen for 24 hours root-growth ceased entirely and was not renewed. Roots which were given 7 per cent oxygen grew about 3 mm. during the first 24 hours, but none subsequently. In the experiments with 10 per cent oxygen, which were continued for 5 days, growth was found to continue for at least 4 days. Thus, in one culture, the following daily amounts of growth were recorded: 19, 8.5, 2.5, and 0 mm. In another culture, however, growth went on throughout the experiment. In this case the total amount was 9 mm. A control, which was run at the same time and at the same temperature, grew 17 mm.

Although the experiments on the bean were few, they point to the need of a relatively high percentage of oxygen for growth of the roots. It is evident, for example, that 10 per cent is a deficiency at the temperature used. This ranks the bean among the species which are least tolerant of oxygen deprivation.

Pisum sativum.

Numerous experiments were conducted on different varieties of the cultivated pea, including the Canada field pea, the Telephone pea, the Yorkshire Hero garden pea, and market-garden pea. In the aeration studies the effect on root-growth, both of a deficiency of oxygen and an excess of carbon dioxid, with the diluting gas argon, helium, or nitrogen, was under observation. Growth in a soil-atmosphere containing helium and argon is discussed elsewhere in this work, and will be referred to only incidentally in this place. The methods employed in the study have been various, although, for the most part, the one mostly used in connection with the investigations was used mainly with the pea also.

Introductory studies on the relation of the oxygen-supply, or at least on the relation of soil aeration, to the rate of root-growth in the garden pea were carried out by comparing the rate of growth in unaerated tubes with the rate of growth in tube cultures through which a stream of atmospheric air was caused to move slowly. The results were negative. No direct change in the rate could be induced by this means.

Following such experiments, seedling peas were grown in tubes of the usual kind and were so arranged that the growth of the root could be under constant observation by means of a horizontal microscope. By such a method the experiments were always relatively short, and the cultures were not subject to temperature control, although the temperature of the cultures or of the air immediately about them was usually observed. In the direct observation tests oxygen in various percentages, between 0.5 and 1.5 with nitrogen, was used. The general results of these experiments can be briefly given. In the gaseous mixtures containing 1.5 per cent oxygen or less, the results were not consistent. In some instances root-growth occurred, but in others it did not. Table 15 summarizes the results of a representative test with 1.5 per cent oxygen with nitrogen.

That, in fact, a certain amount of root-growth may continue in a soil atmosphere containing 1.5 per cent oxygen with nitrogen was observed in other direct-observation experiments also, which need not be further referred to here.

TABLE 15.—*Root-growth of garden pea in 1.5 per cent oxygen.*

Time of observations.	Air-temperature,	Root-growth	
		No. 1.	No. 2.
	° *C.*	*mm.*	*mm.*
11h 45m to 12h 15m p. m.	22	0.3	0.2
12 15 2 15	20.5	.5	.2
2 15 3 15	19	.3	.1
3 15 4 15	16.5	.2	.1
4 15 9 40 a. m.	12	2.5	1.8

Tests made with a smaller amount of oxygen, as with 0.8 per cent, balance nitrogen, gave conflicting results. For example, on November 26, three cultures were given gas from 11h 30m until 3h 10m p. m., temperature between 19.5° and 23.5° C., with the following results: The root of No. 1 increased 0.5 mm., that of No. 2, 0.1 mm., and that of No. 3, 0.1 mm. during this period. Upon being transferred to the thermostats and left connected with the gasometer with 0.8 per cent oxygen for 24 hours, it was found that no further root-growth occurred in any of the cultures.

It will have been noted that the roots were exposed during the 26th, and while under observation with the horizontal microscope, for a period of 3 hours and 40 minutes altogether. On the preceding day, from 2h 45m to 5h 30m, temperature from 21.5° to 16.5° C., the same roots in 0.8 per cent oxygen with helium grew as follows: No. 1, 0.6 mm., No. 2, 0.8 mm., and No. 3, 0.3 mm. Thus in helium, with as great oxygen deficiency as before, No. 1 root grew 0.1 mm. more in three-quarters of the time than when the diluting gas was nitrogen. Further than that, upon being left overnight in the oxygen-helium mixture, root-growth continued in all of the cultures.

In a preceding paragraph the table summarizes root-growth in 1.5 per cent oxygen with nitrogen. When helium was used as the diluting gas quite as good growth was obtained with 1.1 per cent oxygen, as a single test will indicate. Three cultures were observed to give essentially the same reaction, so that root-growth of one only need be given. While in normal soil air, 20.5° to 22.5° C., for 2h 10m, the root grew 1.7 mm. The culture was then given 1.1 per cent oxygen with helium, for 50 minutes, during which time the root grew 0.3 mm. The culture was then left overnight, with the root in the oxygen-helium mixture, when the root grew 2.3 mm. In other tests in which the same amount of oxygen was used, but nitrogen was the diluting gas, the results were not consistent; in some roots growth continued, but in others it ceased.

Experiments with the direct method on peas in which 0.5 per cent oxygen with nitrogen was used gave only negative results.

In the experiment with the direct method, in which, as above stated, the root-growth was measured with a horizontal microscope, the temperature was not under control and, for the most part, the experiments were of short duration. The experiments with peas, which remain to be described, were with thermostats, and usually each experiment extended over several days, the growth being read each day. The experimental atmosphere used

consisted of from 0.5 to 2 per cent oxygen, with nitrogen, argon, or helium, and, also, in several tests, an excess of carbon dioxid, with or without oxygen sufficient for normal rate of growth, was used.

In the anaerobic experiments negative results only were obtained, whatever the diluting gas, when oxygen was wholly removed from the soil atmosphere. The results with 0.5 per cent oxygen were various. In case nitrogen was used as the diluting gas, no growth of the roots took place, either in static or streaming gas, but when helium was used growth occurred not only when the gaseous mixture was streaming, but also when it was static, as given in another place in this study. The temperature of the soil was about 25° C. in these experiments.

Experiments in which 1 per cent oxygen was employed with argon, and with about 1 per cent carbon dioxid, did not give consistent results. Thus, an experiment with 4 cultures was run 48 hours. During this time No. 1 did not grow, No. 2 grew the first day, the root of No. 3 grew 0.5 mm. each and every day, while that of No. 4 grew 0.5 mm. the first day and 1 mm. the second day. When the roots were given 0.5 per cent oxygen growth stopped in all. In later experiments, however, the results were wholly negative. Thus, on April 21, 4 cultures were under observation. The length of the roots was 10, 8, 10.5, and 7 cm. Growth of the roots was demonstrated while in normal soil-air, after which 3 of the cultures were given 1 per cent oxygen with nitrogen for 24 hours. No growth occurred in the cultures which had been given the gas, but the root of the control increased 14 mm. in length during the same time. None of the roots of the plants, which had been kept in the experimental atmosphere, renewed growth upon being aerated. The tips had lost their turgidity. After 2 days it was found that the roots of 2 of the cultures were shrunken for the entire portion in view, but that only the tip of No. 3 had such appearance.

The effect on root-growth of the Telephone pea, of 1.6 per cent oxygen with nitrogen, was observed in an experiment begun May 28. Two cultures were used, of which the root of No. 1 was 10 cm. long and that of No. 2 was 16 cm. long. The temperature of the soil was 27° C. During the 24 hours just before the gas administration the roots grew 31 and 24 mm. in normal soil-air. They were then given 1.6 per cent oxygen for 24 hours, at the end of which time it was found that the root of No. 1 had grown 2 mm., while that of the other culture had not grown. Analyses of the soil-atmosphere of the cultures at the end of the 24-hour period showed that there was between 1.4 and 1.8 per cent oxygen in each.

It appeared, therefore, that although growth of the roots might take place in a soil atmosphere containing 1 or 1.6 per cent oxygen, with nitrogen as the diluting gas, in many instances at least, growth ceased. Precisely what determined growth under such conditions was not learned, although it does not appear unlikely, as will be shown later in this paper, that the temperature of the soil may be an important factor. However that may be, it became of interest to determine somewhat more closely the possible concentration of oxygen, as represented by the percentage, which is consistent with root-growth under all growing temperatures. This was not surely done, but experiments with 2 per cent oxygen in nitrogen indicated, as the following will show, that such must be close to the upper critical percentage. One experiment will be sufficient to illustrate this. On August 2 to 3, and while in normal soil-air, the root of a culture grew 9 mm.; temperature 27° C. The gaseous mixture with 2 per cent oxygen was then given 7 days continuously. From 4 to 8 mm. growth of the root occurred

each of the first 3 days of the test, after which time it decreased and ceased on the seventh day. Analogous results were obtained in other experiments.

A few tests were made with the Canadian field pea on the effect of an excess of carbon dioxid when an adequate amount of oxygen is present. The gaseous mixtures contained between 6.8 and 50 per cent carbon dioxid, but cylinder carbon dioxid was also used.

In a preliminary experiment 3 specimens were given 3 different concentrations of carbon dioxid, but otherwise treated alike. No. 1 was given cylinder carbon dioxid; No. 2, 10 per cent carbon dioxid with atmospheric air. The test was run 72 hours, with the following results: Root-growth ceased in No. 1 as soon as the gas was administered. In No. 3 there was 2 mm. growth the first day and 1 mm. the second day of the experiment and none afterward. But in No. 2, with 10 per cent carbon dioxid, from 4 to 10 mm. growth took place each day.

On January 31 an experiment was set up in which 4 plants were under observation. The soil-temperature of all was the same, about 25° C. The root-growth for 24 hours, while in normal soil-air, was 14 mm., 11 mm., 20 mm., and 5 mm. for Nos. 1 to 4 respectively. Then a mixture consisting of 30 per cent carbon dioxid, 30 per cent oxygen, and the balance nitrogen was given 24 hours. At the end of this time it was found that Nos. 2 and 4 had not grown, but that a growth of 10 mm. had taken place in No. 1 and of 4 mm. in No. 3. The notation was made that there had been an outward leak, but a slow one, in No. 1.

One experiment was planned in which equal parts of oxygen and carbon dioxid were used. This ran 72 hours. In cultures 1 and 2 the gas was not static, but streamed slowly, but in Nos. 3 and 4 it was static. The root of No. 1 grew about 5 mm. daily and that of No. 2, 3 mm. the first day, 1 mm. the second, and none thereafter. In the other cultures, 1 mm. growth was recorded the first day and none after that.

In the experiments above described there was 5 per cent or over of oxygen, and, therefore, sufficient for a certain rate of root-growth. Other experiments were carried out in which the oxygen-content of the gas used was under 4 per cent and the carbon-dioxid content less than 10 per cent. These can be referred to briefly. A test in which the gaseous mixture consisted of 1.7 per cent oxygen, 10 per cent carbon dioxid, with the balance nitrogen and at uncontrolled greenhouse temperature, gave the following results: Three out of four cultures showed a root-growth between 5 and 4 mm. the first day in the gas. On the second day the oxygen-content was reduced to 1.2 per cent, with the result that the growth became between 0.5 and 2 mm. for the first day and between 1 and 1.5 mm. the second day in the mixture. When, however, the amount of oxygen was increased to 2.8 and the amount of carbon dioxid decreased to 6.8 per cent, the growth during 24 hours was somewhat increased. It appears evident that in the experiments, with so small an amount of oxygen, the effects noted may have been attributable to this fact rather than to the relatively small partial pressure of carbon dioxid, which, as was indicated by experiment carrying many times the amount, was not sufficient of itself to greatly depress the rate of growth. As a matter of fact, the experiments as a whole appear to indicate that seedling Canadian pea can maintain a relatively good rate of growth in a fairly large percentage of carbon dioxid, but that it is dependent on a fairly good oxygen-supply. This again is related to the temperature of the soil, as will be presented in a later paragraph.

Potentilla anserina.

Potentilla anserina of these studies was collected in the swampy lower portion of the experimental garden of the Coastal Laboratory at Carmel. Cuttings, with portions of the roots, were transplanted to the glass culture tubes, where both root and shoot growth took place in an apparently normal manner.

The experiments were concerned both with a deficiency of oxygen in the soil-atmosphere and with an excess of carbon dioxid. In the former, gaseous mixtures containing from 0.5 to 2 per cent oxygen with nitrogen were used, and in the latter, various concentrations above 25 per cent.

The introductory experiments were carried on outdoor and without temperature control, but later the cultures were transferred to the greenhouse laboratory and put in thermostats running about 23° C. A portion of the cultures were given 0.5 per cent and a portion 2 per cent oxygen, and, finally, cylinder nitrogen which had been passed through alkaline pyrogallol was given to some of the cultures. The experiment lasted for about 7 weeks.

So far as the results with 2 per cent oxygen is concerned, it can be said in brief that in all instances root-growth took place, and, so far as could be seen, at about the expected rate for the temperature. In 0.5 per cent oxygen, however, the growth of the roots was not consistent. Thus, in some instances, it stopped, but in others there could be seen little difference between the reaction to 0.5 and to 2 per cent oxygen. In any event, however, and especially in long experiments, a certain amount of growth was seen to go on in a soil-atmosphere with the smaller partial pressure of that gas. Thereupon cylinder nitrogen was run through wash-bottles containing alkaline pyrogallol before introducing it into the cultures, but the results remained much as before, as an account of one such experiment will indicate. Thus on September 15, and for one week, such oxygen-free (?) nitrogen was given two cultures in which a slow rate of root-growth had occurred for several weeks. Of these cultures, No. 3 had the following daily measurements for growth while in the soil-atmosphere mentioned, namely 1.5, 1.5, 1, 1, 2, 0, and 0 mm. Those for No. 4, at the same time and under the same cultural conditions, were 2.5 and 3 (two roots), 2.5 and 1 (two roots), 2.5 and 2 (two roots), 4.5, 3.5, 2.5, and 4.5 mm.

The method of sealing which had been used, and the one mostly followed, was to pack plasticine closely about the base of the shoot or the crown of the root. But in *Potentilla* it was at length found necessary to remove the soil from about the crown of the root and pour in melted cocoa-butter or sealing-wax, and then to cover with plasticine. With this done the results were more uniform, as the following sketch of a single experiment will indicate. On August 13, six cultures were prepared for observation and were left in normal soil-air for 24 hours at soil-temperatures of 30°, 27°, and 18° C. Nos. 1 and 2, at 30° C., grew 7 and 7.5 mm. (two roots), and 4 mm.; Nos. 3 and 4, 27° C., grew 8 and 8.5 mm.; and Nos. 5 and 6, 18° C., 6 and 3.5 mm. Then 1.2 per cent oxygen was given all of the cultures for 24 hours, and it was found at the end of the period that there had been no root-growth in Nos. 1, 2, 3, or 4, and only 1.5 and 1 mm. in Nos. 5 and 6.

The cultures were then aerated and root-growth was resumed, but at a somewhat slower rate than before the administration of the gas, and full recovery was only attained during the second 24-hour period of normal aeration.

At the close of the experiment the partial pressure of oxygen was increased to 2 per cent, which was administered 24 hours. The resulting rate of growth was somewhat under that normal for the temperature but fairly rapid, being between 1.5 and 3.5 mm. for temperatures 27° to 30° C., and 2.5 to 4 mm. for the roots at 18° C.

In the second series of experiments with *Potentilla* the reaction of the roots to an excess of carbon dioxid in the soil was observed in several tests. In these plasticine seals were used, but the gaseous mixtures were kept under slight pressure always, so that if there were faulty seals the leaks would be outward. .

In a preliminary experiment the effect of 10 per cent carbon dioxid on the root-growth was observed. Growth in normal soil-air was demonstrated at the beginning of the experiment. The gas was then given for a period of 6 days continuously. As a leading result it can be said, in short, that growth took place in every root while in the mixture, but the rate was somewhat below that to be expected in normal soil-air. However, it varied not a little, both as between different cultures and as between the roots of one and the same culture.

On August 31 an experiment with 5 cultures was set up and was continued for 3 weeks at a soil temperature of 25° C. The mixture used consisted of 26 per cent carbon dioxid with atmospheric air. A growth of from 2.5 to 7 mm. was noted during 24 hours preceding the gas administration. Three of the cultures were then given the gas for two 24-hour periods during the first of which from 0.5 to 1 mm. growth occurred. During, the second day in the gas, also, and throughout the remainder of the test, about the same daily growth-rate was noted. However, whenever open irrigation was practiced, the root-growth for the following 24 hours was usually somewhat more than when irrigation was effected by allowing water to run into the soil from the reservoir in the lower portion of each culture tube.

In the second experiment two cultures were used, the soil-temperature was 23° C., and the mixture contained 48 per cent carbon dioxid. The roots increased 4.5 and 3.5 mm. in length during 24 hours before the administration of the gas was begun. The mixture was then given 72 hours continuously, during which time the cultures were irrigated once. In both the result was the same. Growth of the roots ceased as soon as the gas was given and was not resumed during the balance of the experiment. At its termination, however, the roots were not shrunken, but were normal in appearance.

In a third experiment 50 per cent carbon dioxid was used, also with negative results. Cylinder carbon dioxid was given the roots of 4 specimens, in which growth in normal soil-air had been demonstrated, with the effect, as would be expected, that root-growth ceased.

The tolerance of the roots of *Potentilla* for an excessive amount of carbon dioxid in the soil, therefore, does not appear to be especially marked, but it does not appear to be likely that the amount, which the roots must usually or even occasionally encounter, influences to an important degree root-growth in the species.

Prosopis velutina.

Numerous experiments were carried out on the aeration relations of the mesquite. Seedlings only were used, and were grown from seeds collected in the vicinity of Tucson. The original object in studying the species was based on an attempt to learn whether the reaction of the roots, either to the temperature of the soil or to the oxygen-carbon dioxid partial

pressure in the atmosphere of the soil, had demonstrable relation to the character of the roots as shown by the development in the proper habitat.

The roots of the mesquite, probably more than of any other woody species in southern Arizona, are capable of an exceedingly wide range in adjustment. Thus, they may either penetrate the ground deeply, 5 meters or more, or they may remain close to the surface of the ground, and if so, may extend for a distance of 25 meters, more or less, from the main stem of the plant, and they may assume various positions between these extremes.

Relation to Temperature.

The temperature of the soil at depths taken by the roots of the mesquite is various, differing not only with the seasons but with the depth. Of these differences it will be sufficient to point out that although in winter the soil above 1 meter may be too cold for root-growth, an entire year may pass in which the temperature at 1.2 meters may not be as low as 12° C., and it is not improbable that at a greater depth the minimum may be somewhat higher than this. As to the other extremes, in summer and at a depth of 30 cm., as much as 72 per cent of the time the soil may have a temperature of 30° C., which is not far from the optimum for root-growth in the species. With suitable soil moisture, therefore, it would appear possible that growth of some portion of the root system may take place at any season. So far as the extremes in vertical distribution of the roots is concerned, it would accordingly appear that soil-temperature, at least, may not be a limiting factor. It is probably true, however, that there is a coincidence between the occurrence of the greatest mass of roots and fairly high temperature, taken together with an appropriate supply of moisture.

The great range of adjustment to soil-temperature and the correspondingly great variation in the position assumed in the soil by the roots of the mesquite suggested the possibility that they may react to other definable features of the soil environment as well. Accordingly, as suggested above, a systematic study on the relation of the roots to the oxygen-supply of the soil and to the carbon dioxid in the soil was undertaken. Although the experiments on the aeration relation of the mesquite roots have been very numerous, a summary only of those which appear to be the most significant will be given.

Relation to Oxygen.

In the introductory experiments, several percentages of oxygen, between 0.5 and 13, with the diluting gas nitrogen, were carried out, and also experiments with an excess of carbon dioxid.

In the tests with 0.5 per cent oxygen the results were not consistent, although, as will be seen, they appeared to indicate that growth in so great oxygen deficiency would continue for a limited time. For example, in a typical experiment 3 seedlings were used, with roots measuring between 5.5 and 8 cm. The temperature of the soil was 23° C. The gas administration continued for 6 days. Of this time root-growth was seen to continue for 4 days in one, 5 days in another, and 6 days in the third. The actual daily rate of growth was high. Thus in No. 168 it varied between 20 and 26.5 mm., with 26 mm. the fourth day. In No. 164 the daily growth was between 17 and 25.5 mm., the last-named amount occurring the third day. On the sixth day the root increased 21 mm. in length. However, not all roots observed in so small a concentration of oxygen behaved in this manner. For example, roots Nos. 160 and 161 grew but 2 mm. in 48

hours and none thereafter; and No. 163 grew 3 mm. the first day and continued a low rate for 4 days, when growth ceased entirely.

The length of time the root of *Prosopis* will continue to grow in a soil-atmosphere with so little oxygen is apparently dependent on several things. Of these, the vigor of the seedling is apparently one. In case the initial root-growth is slow the root does not appear to be able to withstand oxygen deprivation well. Although not surely determined in *Prosopis*, it seems possible that the main root, early in the grand period of growth and late in the period, that is, before and after the crest of the growth curve, may have unlike reactions to the concentration of oxygen. Thus, at the time of growth acceleration and before the appearance of laterals, the root may be relatively insensitive to oxygen deficiency, but when the growth-rate of the main root is decreasing, with the formation of laterals, the ill effects of a low oxygen-content of the soil may be especially marked. It is possible, also, had the temperature of the soil been lower than that used in the above experiment, that there may have been sufficient oxygen for a rate of growth commensurate with such temperature, as has been determined in other species, and as was suggested later by other tests on the mesquite. And, again, in so long continued experiments there may have been an inward diffusion of oxygen from the atmospheric air, as is known to occur, by which the amount in the culture tubes was in fact somewhat more than was known to be in the gasometer. However the last may have been, the fact was demonstrated that the root of seedling mesquite is relatively insensible to a great deprivation of oxygen in the atmosphere of the soil. For these experiments, also, it seemed possible that the lower critical partial pressure of oxygen for growth of the roots of *Prosopis* is probably higher than the amount represented by 0.5 per cent.

The rate of root-growth in greater concentrations of oxygen was observed in several experiments. Of these, one series had to do with the effect of 2.6 to 3 per cent. The tests lasted from 5 to 11 days. The roots were from 10.5 to 17 cm. long and the temperature of the soil was about 29° C. In one set of experiments, which ran for 3 days, the rate of root-growth in 2.6 per cent oxygen was approximately one-half the rate in normal soil-air just before the gaseous mixture was given, indicating that, under the conditions of the experiment, 2.6 per cent constituted a deficiency of oxygen. A test was run in which the root was slightly less than 10 cm. long, with 3 per cent oxygen and for 5 days, with the result that growth continued throughout the period. In yet another experiment, with the same amount of oxygen, cultures were used in which the roots were 2.5 to 3 cm. in length. This lasted 11 days. The temperature was not controlled, for a part of the time it reached 34° C. Root-growth continued for the whole time, but at various rates. On the eighth day, when the temperature of the soil was 24° C., the growth was the most rapid. Whatever the length of the root at the time of the test, it appears that with an oxygen partial pressure equaling 3 per cent, the growth during the first day in the gas is always greater than in subsequent days. A single experiment will be sufficient to show this. On July 7 an experiment was set up with 4 cultures, Nos. 100 to 103. The initial root-length of the 4 was 80, 50, 47, and 40 mm. Cultures 100 and 101 will illustrate the extremes in the results. The root of No. 100, in 3 successive days, grew 23, 6, and 2 mm., and that of 101 grew 21, 15, and 12 mm. The temperature of the soil was 30.5° C., or optimum for root-growth. That the rate the first day in the gas was one-half or less than one-half the expected amount at the temperature is evident, and again points to the fact that 2.6 per cent, the percentage used, constitutes, for the temperature, a factor limiting root-growth.

A few experiments were carried out with about 5 per cent oxygen. The leading results may be summarized. The tests lasted from 3 to 8 days. In a typical instance a root-growth of 23, 12, 11, and 3.5 mm. was recorded for as many successive days. In this instance the root was 5 cm. at the beginning of the experiment, so that the decrease in rate may have been associated with the passing of the grand period. After the grand period of root-growth it appears that the rate of growth is relatively slow. For example, in a soil-temperature containing about 5 per cent oxygen and at a favorable temperature for growth, the root of one culture increased only 10 mm. in 96 hours. However, there are evidently other conditions to be taken into account other than shoot-development or the stage of development of the root. For example, in a culture where cotyledons only were present, and during a period of the same length, the growth of the root was only 7 mm.

In studying the possible relation between shoot-development and root-growth, seedlings with different-sized cotyledons were used. Measurements were made both of the cotyledons and of the hypocotyls, in relation to root-growth. As developed under normal conditions, the cotyledons of *Prosopis* attain a size about 14 by 18 mm. and the hypocotyl reaches various lengths. Cultures were set up in which 5 per cent oxygen was given the roots, and it was found that the cotyledons did not get to be so large as would be expected. The largest measured about 9 by 11 mm. In this instance the hypocotyls were between 30 and 31 mm. long and the roots between 23 and 38 mm. in length. The experiment lasted 8 days. In no instance where there was an oxygen deficiency in the soil was it seen that the cotyledons got to be of normal size, or anything near it. The hypocotyl, on the other hand, is apparently not directly affected by such diminution in the oxygen-supply as in these tests.

Some experiments were carried out with 7, 10, and 13 per cent oxygen, the idea being to determine the upper limits of oxygen deficiency for root-growth in *Prosopis*. A typical experiment was conducted in which the soil-temperature was 21° C. and in which the gas administration was continued 5 days. The daily root-growth of No. 156, which is representative, in 7 per cent oxygen was 14, 14, 8, 6, and 3.5 mm. When compared with the expected growth at the same temperature, and under normal aeration conditions, it was found to be approximately 30 per cent under normal.

In 10 per cent oxygen the rate of root-growth was about as rapid as in 7 per cent. As an example, the behavior of No. 170 can be cited. During 6 days in the gaseous mixture the root of this plant grew 14, 13.5, 10, 5, 4.5, and 2 mm.

Finally, tests were carried out with 13 per cent oxygen in which the mixture was given continuously for 11 days. The temperature of the soil was 25° C. The main results are summarized in table 16.

It will be seen that in 2 of the cultures, of 2 plants each, 2 roots were measured. The same was true of the culture with air, although the growth of one only is given. In this experiment the gaseous mixture was changed twice daily, when each culture was irrigated. The results, therefore, were probably not affected by the number of roots in each culture. The decrease in rate of growth is probably to be associated with the appearance of laterals, as in other instances, although this was not especially noted in the experiment last described.

In the experiments with 5 to 13 per cent oxygen the temperature of the soil was about 25° C. It was shown that the behavior of roots in such concentrations of oxygen, and at that temperature, was not far from what

it would be in normal soil-air. It can not be concluded, however, that 5 per cent oxygen, for example, is the upper limit of oxygen deficiency in the mesquite, for the reason, as will appear shortly, that at temperatures near optimum the oxygen requirement of a species is greater than at soil-temperatures which are low or relatively low.

TABLE 16.—*Root-growth, Prosopis velutina, 13 per cent oxygen.*

	No. of culture.				
	213*a*	213*b*	215*a*	215*b*	[1]218
Root-length at beginning of experiment	*cm.* 3.5	*cm.* 7.5	*cm.* 7	*cm.* 5	*cm.* 7
Growth:	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
First day	11.5	11.2	20	22	....
Second day	15.5	11	23	20	10.5
Third day	14.5	10.5	28	26	20
Fourth day	9.5	11	27.5	22	24
Fifth day	11	10.5	14.5	18	22
Sixth day	7	5	13.5	8	8.5
Seventh day	8	5	6	7	12
Eighth day	7	5	4	6	12
Ninth day	8	6	1	5	7
Tenth day	5	3.5	0	1	7
Eleventh day	2.5	1	0	0	7

[1] Root in normal soil-air.

In later experiments especial attention was given to the temperature and to the oxygen-content of each culture, and, for the most part, only low concentrations of oxygen were used. Other features were held in mind as well.

TABLE 17.—*Root-growth, Prosopis velutina, in 0.6 per cent oxygen.*

	Soil temperatures.								
	20° C.		21° C.		21° C.	16° C.		16° C.	
	Culture.		Culture.		Culture.	Culture.		Culture.	
	1A	1B	2A	2B	3	4A	4B	5A	5B
Root-length at beginning	*cm.* 8.7	*cm.* 4.3	*cm.* 7.3	*cm.* 5.4	*cm.* 8	*cm.* 7.5	*cm.* 5.5	*cm.* 7.	*cm.* 7.
Growth:	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
First day	25	15	15	6	10	5	4	5	4
Second day	0	0	0	.5	.5	0	.5	0	1.5
Third day	0	0	..	0	2.5	0	2.5	1.5	2.5
Fourth day	5	0	6.5	0	6	0	1	2	2
Fifth day	7.5	..	6.5	0	6	0	1	2.5	5.5

Representative results, to be found in table 17, with 0.6 per cent oxygen at different soil-temperatures were had in a test begun March 12. In it are given the root-length at the outset, the growth during the first day when the roots were in normal soil-air, and the fourth and fifth days, when they were aerated.

In the experiment 2 plants were grown in each tube, except in No. 3, where there was but 1. The gaseous mixture was given in the morning of

the second day, and it was noted that root-growth did not stop immediately, but that it continued for about an hour after the beginning of the administration of the gas. The table of growth refers to the behavior of the root after the lapse of about an hour following the beginning of the gas administration, that is, after the possible readjustment of the soil system to the diminished supply of oxygen. It is to be noted, however, that in experiments which continue for so long a time as many of those mentioned in this paper, it does not appear to be necessary to take this period into account. In the present instance the significance of the reaction to 0.6 per cent oxygen is especially marked the second day, the third of the experiment, when growth at the lower temperatures only is seen to take place. The rate of supply, with 0.6 per cent oxygen, at 16° C., is thus sufficient for a rate of growth between about 21 and about 51 per cent normal, whereas at 29° C. there is no growth. Although the root-length of the cultures was between 4.3 and as much as 8.7 cm. there were no laterals, which excludes an important factor in relation to growth variation.

On March 1 an experiment was set up in which 1 per cent oxygen was used. The oxygen content of each tube was determined at the beginning and also on each of the 2 days the roots were in the gaseous mixture. The leading data derived from the experiment are summarized in table 18, in which duplication of results is omitted.

TABLE 18.—*Root-growth, Prosopis velutina, in 1 per cent and 1.6 per cent oxygen.*

	Soil temperature.			
	29° C.	23° C.	21° C.	21° C.
	Culture 1.	Culture 2.	Culture 3.	Culture 4.
Root-length at beginning	*cm.* 9	*cm.* 8	*cm.* 8	*cm.* 6.5
Growth:	*mm.*	*mm.*	*mm.*	*mm.*
First day	30	15	11.5	8
Second day	2	3	1.5	4
Third day	0	1.5	1.5	5
Fourth day	10.5	8	6	8
Fifth day	4.5	4.5	1	5.5
Sixth day	0	....	2	4

As a leading result, it will be seen that at 29° C. root-growth does not continue the second day in 1 per cent oxygen or in 1.6 per cent, although at 21° C., as well as at 23° C., it goes on slowly. During the first day in the mixture, however, root-growth at 29° is fairly active. It seems probable that while 1.6 per cent constitutes a deficiency of oxygen at the highest temperature used, possibly it is not far from the upper critical percentage for 27° C. It is evident also that for 29° the upper critical concentration of oxygen is greater than 1.6 per cent for *Prosopis*, while it may be about 1.6 per cent for 16° C. On the other hand, at 16° C., as above indicated, the lower critical oxygen partial pressure is under 1 per cent. Thus, within the temperature range for root-growth in *Prosopis* the critical percentage of oxygen probably varies more than 1 per cent.

GROWTH IN CARBON DIOXID.

In the experiments with excessive amounts of carbon dioxid, seedlings of *Prosopis* were used as in the experiments with an oxygen deficiency,

and the tube method also was employed. The concentrations of the gas were between about 25 per cent and that represented by the cylinder gas, that is, about 97 per cent. The gas was diluted with atmospheric air, for the most part, with the effect that as the carbon dioxid increased in amount the oxygen partial pressure decreased. But always, unless otherwise stated, the latter was present in sufficient amount for root-growth in the mesquite, although, clearly, in some instances, there was a deficiency at certain temperatures. However, no difficulty was experienced in determining effects of an excess of carbon dioxid as opposed to those following the use of small percentages of oxygen.

The experiments were carried out in two general methods. In one, the first to be mentioned, the exposure of growing roots to an excess of carbon dioxid was for short periods, and also, the percentage was usually high. A leading point observed in this case was the length of time following the exposure to an excess of carbon dioxid necessary to recovery and the fact of recovery. The other method had to do with longer exposure-time; and in this case survival and growth-rates were the points especially noted. In both instances, and as in the experiments with oxygen, no attempt was made to duplicate possible aeration conditions of the soil, as they are not sufficiently well known for this purpose.

The method of procedure used in the introductory tests was either to record the amount of growth in unit time, while the root was in normal soil-air, and then give the gaseous mixture, after which growth in normal soil-air was again observed, or the first observations were omitted. In any event, the length of the gas administration was usually short, as was, in consequence, the duration of each test.

In the experiments with 25 per cent carbon dioxid the roots were exposed from 2 to 4 hours continuously, and the amount of growth was determined for each half-hour. The soil-temperature was 27° C. The following readings of growth are of a typical experiment and illustrate sufficiently well the reaction of roots to such concentration of the gas. The half-hourly growth increments were 0.4, 1.7, 1.6, 1.8, and 1.2 mm. From what has already been presented regarding the normal rate of root-growth in *Prosopis* at 27°, it is evident that 25 per cent carbon dioxid, with about 18 per cent oxygen, does not greatly influence its rate for the period and temperature used.

In tests with 40 per cent carbon dioxid with atmospheric air, the administration was continued 2 hours, after which the cultures were aerated, and the roots were under observation for 3 hours while in normal soil-air. As to the leading results, it can be said that the rate of growth of the roots was sharply decreased, but growth did not wholly cease. Several experiments were carried out in which 50 per cent carbon dioxid was used and in which the exposure to the gas varied between 2 and 15 hours. In a test when the gas administration was somewhat more than 2 hours, a root-growth of 0.6 mm. was seen to have occurred. Then growth ceased for about 2 hours while in normal soil-air, and was not renewed until between 2 and 4 hours following the gas administration. But one additional experiment need be mentioned. In this the administration was continued 15 hours at a soil temperature of 31.5° C. As a result, it was found that growth of the root continued slowly for about one-half hour and then ceased.

In higher percentages also of carbon dioxid growth was found to continue for a short time, less than an hour, in the mixtures employed, and to recover in normal soil-air, provided the exposure to the gas had not been overlong. However, the reactions are influenced by the temperature of the soil, as

will appear in the following account of a few typical tests. The leading results can be presented briefly. In 75 per cent carbon dioxid growth ceases within an hour, but, also, it is renewed within an hour after the gas has been replaced by atmospheric air. With 85 per cent the length of the exposure time varied between 1 and 6 hours, and the period of quiescence following the gas administration was especially taken into account, as was the temperature as well. Thus, in one experiment, in which the gaseous mixture was given one hour, at a soil temperature of 32° C., the root did not cease growing, but the rate was greatly lowered, and recovery took place only after about 4 hours in normal soil-air. Another experiment was run at a soil-temperature of 19° C., in which the gas administration lasted 2 hours. Root-growth ceased at the beginning of the administration and was not renewed until 2 hours after atmospheric air had replaced the 85 per cent carbon dioxid. In the experiment in which the roots were given 85 per cent carbon dioxid for 4 hours, the soil-temperature varied between 20° and 31.3° C. during the first of the test and fell to 28° at its close. The growth of the root, at 2-hour intervals, was as follows: 0.5, 0.4, 0, and 0.4 mm. The first two readings were made during the gas administration. Partial recovery thus occurred the fourth hour in normal soil-air following the gas administration. In the last set of experiments with 85 per cent carbon dioxid, the gas was administered for 6 hours continuously. In one test, which only need be referred to, the root continued to grow throughout the entire administration period and made quick recovery afterward. The following measurements were made, at 2-hour intervals, while the root was in the gas: 0.1, 0.1, and 0.2 mm., and during the first 2-hour period following the administration the root grew 0.5 mm. In other experiments the results were somewhat different, but agreed in that a certain amount of growth took place for at least a portion of the exposure-time, and that recovery was observed comparatively soon after atmospheric air had replaced the gas used. A few tests were made of the immediate effect of cylinder carbon dioxid, that is, of approximately 97 per cent carbon dioxid, and the length of the gas administration was between 15 minutes and 3 hours. The results can be stated briefly. When the gas was used 15 minutes the growth-rate was immediately depressed or growth ceased entirely. In any event, recovery of the normal rate of growth did not take place sooner than one hour following the replacing of the gas with atmospheric air. These results were obtained at soil-temperatures between 27.5° and 31° C. The effects of cylinder carbon dioxid were apparently connected in some way with the soil-temperature. Thus, in one test, soil-temperature 26° to 28° C., the rate of root-growth fell 50 per cent during the hours in which the gas was given for 30 minutes, and 2 hours elapsed after the root was in normal soil-air before the rate was normal for the temperature. At a soil-temperature of 31.3° C., however, a normal rate of growth occurred during the first hour following the replacement of the carbon dioxid with atmospheric air. Upon repeating the experiments with soil temperatures at about 27° and 32° C., analogous results were obtained. In these experiments the length of the root did not seem to matter. The root 6.5 cm. long reacted quite as the one from 17 to 22.5 cm. in length. In experiments where cylinder carbon dioxid was given 60 minutes, the results were about as in the 30-minute administration, save for the longer time required for recovery. Where the administration period was increased to 3 hours, growth of the roots ceased, probably during the first 30 minutes or less, and recovery did not take place for over 3 hours after the gas had been replaced by atmospheric air.

The experiments with a great excess of carbon dioxid in the soil indicated that even where the partial pressure is far and beyond what probably occurs in natural soils, and where the root of *Prosopis* is exposed to such gas for 3 hours or less, no permanent injury takes place. Moreover, the ill effects of the gas is apparently less at temperatures near optimum for root-growth in the species than at those which are relatively low.

Salix lasiolepis.

The experiments on the aeration relations of *Salix lasiolepis* were carried out on cuttings which were obtained from trees growing on the borders of a stream in the lowest portion of the experimental garden at Carmel. Adjoining the stream is a small swamp in which, among other species, occur *Juncus effusus* var. *brunneus* and *Potentilla anserina*, the aeration relations of which are reported upon elsewhere in the present study. So far as can be told by superficial examination, the habitat of the willow may be somewhat better aerated than that of the other species, because the stream is perennial and its gradient is relatively steep. Even with these conditions, which forward gaseous exchange in the contiguous soil where the willow roots lie, the soil is probably poorly aerated, at any rate as compared with that of the habitat of *Eriogonum nudum* and of *Stachys bullata*, of which the relation to oxygen and to carbon dioxid is also referred to in this paper. In fact, at certain seasons the soil of the willow habitat must be poorly aerated, even if at others, as in summer, such may not be so much the case.

The experiments were carried on partly in the constant-temperature chamber of the greenhouse laboratory at Carmel, where, at the time, the temperature varied but little from 18° C., and in part in the greenhouse laboratory itself. In the latter case the temperature of the soil was kept about 23° C.

The gaseous mixtures used in the tests comprised 0.5 per cent oxygen with nitrogen; 2 per cent oxygen; oxygen-free nitrogen; 45 and 50 per cent carbon dioxid with atmospheric air; and cylinder carbon dioxid. Two series were carried out, of which the first had to do with the reaction to a deficiency to oxygen and the second with the effects of an excess of carbon dioxid.

An introductory experiment was run in which the roots of the willow were exposed first to normal soil-air, then to 0.5 per cent oxygen with nitrogen, and finally to normal soil-air again. Growth of the roots was demonstrated under normal aeration conditions, at least in aerated soil, but when the gas nearly devoid of oxygen was given, it promptly ceased. Upon the admission of atmospheric air, however, it was renewed. The dependence of the roots of the willow on a certain amount of oxygen in the soil, therefore, appeared to be indicated by these results.

In the second experiment of the series other results were obtained. In this series the time was extended to 21 days. Cylinder nitrogen, containing 0.5 per cent oxygen, was kept in rubber balloons, and constant connection was made with the cultures, with the effect that in each the experimental atmosphere was under slight pressure. The gas in the balloons was renewed frequently to prevent, so far as possible, contamination through inward diffusion of oxygen of the surrounding air. That such occurred, however, will not appear to be unlikely from the results of the experiments. Irrigation was affected by up-ending the culture tubes. This experiment was carried out in the constant-temperature chamber of the laboratory. As the leading result, it can be stated briefly that a growth of between 2 and

3 mm. was observed to occur each day. Root-growth was thus demonstrated to take place in cylinder nitrogen, provided balloons were used to store it, and, although it was not definitely shown that the soil-atmosphere contained no more than 0.5 per cent oxygen, it yet was apparent that the growth of the roots would continue in a very great deficiency of that gas and at the temperatures employed.

The effect of 0.6 per cent oxygen on root-growth in the willow was observed in an experiment carried on August 24 to 27 under the following conditions: In the cuttings mostly used, the ends of the cuttings, which were somewhat dried, were left freely exposed to the atmospheric air on the one hand and to that of the soil on the other, with the possibility that diffusion of oxygen from the surrounding air might have taken place through the old wood, by which the amount in the culture would be increased. Accordingly, to test this possibility in the present experiment the exposed ends were covered heavily with grafting-wax. Immediately before the experiment began the root was seen to grow 7 mm. in 24 hours at a soil temperature of 20° C. The gas was then given continuously for 72 hours, during which time no growth of the root took place. Determination of the amount of oxygen present on the morning after the first 24 hours in the gas showed that all in the culture tube had been removed by the roots. The tip of the root was found to be shrunken in a zone about 3.5 mm. from the root-cap. Aeration of the root failed to bring about the renewal of growth or a return of turgescence of the tip of the root.

In an experiment carried out July 19 to 22, the rate of root-growth at a soil-temperature of 30° C. and in 2 per cent oxygen was observed. In this instance also the ends of the cutting were sealed with grafting-wax and the culture itself was sealed with grafting-wax and plasticine. Just before the gas was administered a root-growth of 8 mm. in 24 hours was demonstrated. Then the 2 per cent oxygen with nitrogen was given for 24 hours, with the result that the rate of growth was cut to 1.5 mm. The culture was then aerated for 24 hours. Some growth took place during this period, but the tip of the root turned and was lost to view. Thus the direct influence of temperature on oxygen requirement in the willow, which will be discussed in another section, was definitely established.

Following the experiments mentioned, others were planned in which an attempt was made to remove all of the oxygen from the experimental gas, and to keep it from diffusing into the cultures through the seal or otherwise, during the course of the test. In order to effect the former, the cylinder nitrogen was passed through wash-bottles containing alkaline pyrogallol in the first of the anaerobic experiments, and through a reducing electric furnace in the later ones. The results of the two types of experiments will be outlined separately.

In the first anaerobic experiment cultures Nos. 1 and 2 were used. In this the rate of root-growth was observed in normal soil-air, then in the experimental gas, and finally after having been aerated. Two roots were measured in each culture. For a period of 6 days immediately preceding the gas administration the root-growth was as follows: In No. 1 it was 33 mm. for each root, and in No. 2 the total growth of one root was 32.5 and of the other 50 mm. Immediately after growth of the roots in soil-air had been demonstrated, the cultures were given cylinder nitrogen, which had been passed slowly through three wash-bottles containing alkaline pyrogallol. The test lasted 24 hours. At the end of this period it was found that root-growth had not taken place in No. 1, but that in the other culture growth had occurred. This amounted to 1.5 mm. for one and 4 mm. for

the other root. These inharmonious results pointed to a possible leakage in No. 2, which was made probable from the fact that, when the cultures were aerated, the growth during the subsequent 24 hours in both specimens was 4 and 5 mm.

In the next experiment the cylinder nitrogen was passed both through alkaline pyrogallol and an electric reducing furnace before it was used in the cultures. At the outset cylinder nitrogen was used to replace the soil-atmosphere, and then a relatively large amount of the oxygen-free nitrogen was given the cultures. Finally, the gas was put under slight pressure and connections were maintained with the gasometer. A control was run parallel with the specimens used. The experiment lasted 72 hours. At the expiration of that time it was found that the root of the control had increased 10 mm. in length, but that the roots in oxygen-free nitrogen had not grown. It appears, accordingly, that the growth of roots in cuttings of the willow is dependent on a certain even if a small amount of oxygen in the soil.

The second series of experiments on the willow was on the growth-rate of the roots in an excess of carbon dioxid. In the series 45 per cent carbon dioxid with atmospheric air, equal parts carbon dioxid and oxygen, and cylinder carbon dioxid were used.

In the first experiment, with 45 per cent carbon dioxid, the mixture was given continuously for 4 days. During this period the total growth of the root observed was 14 mm. Then the culture was aerated and root-growth measured for 2 days in normal soil-air. The first day the growth was 3.5 mm. and on the second day it was 6.5 mm.

In the second of the series the oxygen partial pressure was over twice that normal to well-aerated soil, as the percentage of that gas equaled the amount of carbon dioxid. The experiment lasted 72 hours, and during this time a root-growth of 6 mm. was seen to occur.

From these two experiments with an excess of carbon dioxid, and with or without an excess of oxygen, the rate of root-growth was found to be surprisingly high, nearly if not quite equaling that in normal soil-air.

In the third experiment cylinder carbon dioxid was used, in which, as previously remarked, there is about 3 per cent oxygen. Even in this experiment, therefore, the amount of oxygen present is apparently sufficient to allow normal growth of the roots. Root-growth in normal soil-air was demonstrated in the cultures just before the administration of the gas, following which it was given for several hours. The immediate effect was to inhibit root-growth, and to prevent its renewal while in the gas. In this instance the growth of the shoot also was seen to cease with the administration of the high percentage of carbon dioxid.

Stachys bullata.

Stachys bullata occurs in sandy soil at the Coastal Laboratory, Hence the roots are well aerated at all seasons. Plants derived from stolons or from cuttings were used.

The observations of *Stachys* included studies on the reaction of the roots, both to a deficiency of oxygen and to an excess of carbon dioxid. In the former, cylinder nitrogen, carrying 0.5 per cent oxygen, was used. The mixtures used in the experiments with large amounts of carbon dioxid were of two compositions, namely, one in which 45 per cent, with the balance atmospheric air, was used, and one with 80 per cent carbon dioxid, 10 per cent oxygen, and 10 per cent nitrogen. In the last-mentioned series, therefore, oxygen was present in sufficiently large amount to permit normal growth under the usual conditions of temperature.

The experiments with an extreme deficiency of oxygen were carried out for 96 hours. Observations on growth of the roots in normal soil-air showed that in one and the same specimen the daily amount might vary between 3 and 11 mm. at 26° C. Growth of the roots was demonstrated immediately before the gas administration, but as soon as this was begun growth ceased in all of the roots and was not resumed during the remainder of the experiment. The gas was static.

In the second experiment with the small amount of oxygen in the atmosphere of the soil the plants were observed for 7 consecutive days. During the first 2 days the gas streamed slowly through the culture, but for the balance of the experiment it was static. As a result it was seen that a daily growth of 3 and 4 mm. took place the first 2 days, but as soon as the gas became static root-growth ceased. Growth was not resumed during the balance of the test.

In the series with an excess of carbon dioxid, 6 cuttings of *Stachys* were used and a single cutting of *Salix lasiolepis*. All of the specimens were given a mixture which was static, consisting of 45 per cent carbon dioxid with the balance atmospheric air. The test was carried on 4 days and the temperature of the soil was kept at 26° C. Root-growth in all of the cultures was demonstrated just previous to the administration. In the mixture root-growth ceased at once in case of 2 of the *Stachys* cultures, and was only 0.5 to 1 mm. in the others. But the root of *Salix*, during the test, increased in length 1.5, 4, 4, and 4.5 during each 24 hours. All of the cultures were aerated at the end of the fourth day, and it was found that in case of *Stachys* little or no root-growth occurred the first 24 hours in normal soil-air. During the second day, however, the roots grew about 4.5 mm. In *Salix* it was found that growth the first 24 hours after aeration was about as in the carbon dioxid, but during the second 24 hours it was 6.5 mm.

After having been kept in normal soil-atmosphere for 72 hours, the specimens last referred to were given 80 per cent carbon dioxid with 10 per cent oxygen and nitrogen. The result was that, with the exception of one culture in which outward gas leak developed, no root-growth occurred. The root-tips were seen to be somewhat discolored at the close of the test. A single specimen of *Juncus* was given the same treatment as *Stachys* and *Salix*, and in this a very slight growth took place. Following the use of the gaseous mixture described, all of the cultures were aerated and left in normal soil-air. The roots did not recover during the first 24 hours under such normal conditions.

Triticum vulgare.

In the experiments with the cultivated wheat the seeds were planted directly in the tubes and the necessity of transplanting was done away with. The experiments had to do only with reaction to a deficiency of oxygen in the soil. The temperature of the cultures was maintained at about 23° C., by which is meant that it did not vary from this over 1° C. The gaseous mixtures employed were 0.5 and 1 per cent oxygen with nitrogen and 1.9 per cent oxygen with helium.

For the most part, the experimental atmospheres were used statically and under slight pressure, although in one or two experiments, which will be noted, the gases were slowly streaming.

During the course of the experiments, which lasted about 3 weeks, one and the same root of any culture was not measured throughout the test, but different roots of one and the same culture were measured. The reason for this, as will be seen below, is that occasionally a root would cease growing and another and younger root would appear and be in condition for study.

There was thus a certain amount of adjustment and accommodation of the root system as a whole to the oxygen deficiency. However, whether the growth cessation was directly related to the fact of the formation of the newer roots, and thus the normal manner of development, was not determined.

In the first experiment 7 seedlings were used. Root-growth in 4 of these was demonstrated while they were under normal aeration conditions. They then were given cylinder nitrogen for 7 consecutive days. Owing to a certain degree of individuality, the history of the root reaction in a portion of the cultures will be given in some detail.

The longest root of culture No. 1, while in normal soil-air, increased 12 mm. in 24 hours at the beginning of the test. It was then given cylinder nitrogen, which contained 0.5 per cent oxygen, for 7 days continuously. The mixture was static throughout the experiment. The growth was 3, 0, and 0.5 mm. for the first 3 days and none after that. At the end of the period the culture was aerated and root-growth was renewed.

In culture No. 2, at the beginning of the test, the main root grew 6 mm. The culture was then given 0.5 oxygen for 7 days, during a portion of which the gaseous mixture was streaming slowly, bringing fresh gas to the roots of the plant and probably removing carbon dioxid. For a portion of the period, however, the mixture was static. During the first 24-hour period, when the gas was static, the main root increased only 1 mm. in length. On the second day of the test the growth was somewhat more, and during the third day, when the gas was moving slowly through the culture, a growth of 12 mm. was recorded. Two or three roots in each culture were measured on the fourth, fifth, and sixth days of the experiment on culture No. 2. Of the roots measured, one grew 3.5 mm. the fourth day and none after that; another grew 2 mm. the fourth day and 8 and 9 mm. the fifth and sixth days, when a slight leakage was detected in the culture. On the two following days the root-growth was 12 and 13 mm., while, it should be noted, the gas was streaming slowly through the soil. In another root the growth continued 48 hours in the gas and then ceased.

In culture No. 3 several roots also were under observation, either at the same time or at different times during the course of the experiment. With normal soil aeration at the beginning of the experiment, and during a period of 24 hours, a root-growth of 20 mm. was noted. For 72 hours following this, and while in 0.5 per cent oxygen, the daily growth of the roots was 5, 1.5, and 0 mm. The gas was static throughout this period. The culture tube was opened on the fourth day but closed immediately, and the administration of the 0.5 per cent oxygen was continued. During this day, the fourth of the experiment, when the tube was opened, a root-growth of 8.5 mm. was observed in the 24 hours, but during the next succeeding 24 hours the growth was only 0.5 mm. or there was practically none. From this, the fifth day, and for 5 following days, two younger roots were observed while in the same experimental atmosphere, that is, 0.5 per cent oxygen with nitrogen. The roots replaced the older roots in which growth did not take place after the first 48 hours in the atmosphere with little oxygen. The growth of the two roots in question during the last 5 days of the experiment was from 6 to 11 mm. daily.

The main root of culture No. 4 during 24 hours and under normal conditions of soil aeration increased 13 mm. in length. The culture was then given 0.5 per cent oxygen for 9 days continuously. At the beginning only one root was in view. This was 23 cm. long. During the first 24 hours in the 0.5 per cent oxygen the root grew 2 mm., but none subsequently,

although it remained normal in appearance. On the seventh day of the experiment two fresh roots came into view, and for the remainder of the experiment made a daily growth of from 5 to 13 mm.

The history of the growth of the roots of culture No. 5 was about the same as that of No. 4. In both cultures the gas was static.

In culture No. 6 the gaseous mixture streamed very slowly throughout the experiment, during which time the daily root-growth was from 7 to 15 mm.

At the beginning of the experiment the main root of No. 7 was 29 cm. long. Growth of the root ceased with the administration of the 0.5 per cent oxygen and was not renewed during the balance of the test. On the seventh day of the experiment a root 6 mm. in length was placed under observation. This short root increased 6 mm. on the eighth day and slightly on the ninth.

The experiment on wheat was reset at the close, and cultures 1 and 7 were aerated, left in normal soil-air for 96 hours, and growth was noted in the roots of each. The remainder of the cultures were given 0.5 per cent oxygen for the same length of time. In Nos. 2 and 4 it was slowly streaming, but in the balance it was static. The daily growth of the roots of No. 2 was between 6 and 20 mm., and that of No. 4 was from 6 to 17 mm. In the case of cultures 3 and 5, however, with static gas, the growth during the first 24 hours was 0.5 and 2 mm. and none thereafter during the experiment; but when, at its close, these cultures were aerated, root-growth in both was immediately resumed.

After a short lapse of time, in which all of the cultures were aerated, 1 per cent oxygen was administered for 96 hours. Daily examinations revealed the fact of growth in all of the roots which had been measured. In 3 of them, however, the tips of the roots turned away from the direction they had been taking and were in part lost to view. Upon the admission of atmospheric air, however, such roots turned downward and resumed the direction of growth previously taken.

In the final experiment, in which 1.9 per cent oxygen was used with helium, it is sufficient to remark that growth was observed in all cultures used, although the amount was various, and usually not large.

It will be seen, therefore, that although the roots of wheat seedlings are able to grow at a slow rate in 1 per cent oxygen, if static, and in 0.5 per cent oxygen, if slowly passing through the soil, they, however, very clearly require a certain amount of oxygen for growth, which is about 1 per cent. That such partial pressure is close to the minimum for wheat is suggested by the loss of the capacity of reacting to gravitation, as was seen in roots growing in a soil-atmosphere of this composition. A certain degree of accommodation of the root system of the wheat seedling to a soil-atmosphere containing little oxygen was demonstrated, although roots which had developed under "normal" conditions of aeration did not appear to be able to maintain growth, or in some instances to survive, in a soil-atmosphere where the partial pressure of oxygen was extremely low, as 0.5 per cent, with nitrogn as the diluting gas.

Zizyphus lotus.

A single experiment was carried out on the Algerian jujube, *Zizyphus lotus*, which occurs in the dayas of southern Algeria.[1] The seeds from which the culture used was derived were procured at the Daya of Tilrempt and were germinated at the Coastal Laboratory. The soil-temperatures of the experiment were 22° to 24° C. and only 4.5 per cent oxygen with

[1] For the general conditions of the growth of the species, see *Botanical Features of the Algerian Sahara*, W. A. Cannon, Carnegie Inst. Wash. Pub. No. 178, p. 32, 1913.

nitrogen was used. At the beginning of the test the root of the culture was 7.5 cm. long. In the experiment the rate of growth in normal soil-air, in the gaseous mixture, and finally in soil-air was observed. The results can be given in a word. During 72 hours in normal soil-air the root increased 14 mm. in length. Then 4.5 per cent oxygen was given for an equal length of time, but no growth of the root took place. Finally, the culture was aerated, but root-growth was not renewed.

It should be remarked that although there was but one culture and one test, the specimen was quite normal in appearance, and that in the absence of results to the contrary it may be suggested that the species is intolerant of poor soil aeration, at least so far as this refers to a small percentage of oxygen.

Zea mays.

Numerous aeration experiments were carried out on a garden variety of sweet corn (Golden Bantam) and field corn (Early Mammoth), in which the tube method was employed. Although they included a few with an excess of carbon dioxid, for the most part they were confined to studies on the effect of small percentages of oxygen with the diluting gas nitrogen, or, in some instances, helium. The cultures were kept at variouus soil temperatures and the usual course of procedure with the tube method was followed. It can be premised that some difficulty was experienced in the tests on corn. The seeds were either put directly in the tubes or, for the most part, they were caused to germinate in moist filter-paper and then transferred to tubes. At the outset of each test the roots grew well, but they often remained away from the lower and inner surface of the slanting tube, so that it was impossible to follow their course and hence to measure their progress at intervals. Moreover, adventitious roots may form easily, or laterals of the larger roots may arise, or secondary roots may be formed, so that the growth of the root first measured may be modified in ways and by causes possibly not directly related to aeration. As to adventitious roots, they may often be found to develop in the zone of the seal and to penetrate for some distance into the seal itself. Indeed, the behavior of the corn is so different from that of any other of the grasses studied that it should receive especial attention, and possibly special technique should be developed for the study of the aeration relations.

Root-growth in seedlings of the Golden Bantam sweet corn appears not to go on in extreme deficiency of oxygen. This was shown to be the case in an experiment of September 16 to 20 and in others. Thus the root of a culture, while in normal soil-air, increased 7 mm. in length, September 16 to 17, at the temperature of the greenhouse. Cylinder nitrogen, with 0.5 per cent oxygen, was given continuously for the succeeding 3 days, with a soil temperature of 23° C. During this time 1 mm. growth was recorded for the first day and none after that. In another experiment, when similar results were obtained, the root grew only about 12 hours or less in one culture and not at all in another. As is pointed out in another part of this study, when the diluting gas is helium, in place of nitrogen, however, a certain rate of growth of the roots will go on in 0.5 per cent oxygen. In both instances the gas was static.

In an experiment from November 11 to 15, 5 seedlings were given 1 per cent oxygen with nitrogen. At the beginning of the experiment the root in each instance, and there was but one in each culture, was between 2 and 4 cm. long. When the experiment was ended and the roots carefully removed from the soil, it was found that none of the main roots had increased in length. In two of the cultures, however, laterals had been formed and had grown slightly. It seems probable, therefore, for the soil-temperatures

used and with nitrogen as the diluting gas, that 1 per cent oxygen is close to the lower critical percentage for the sweet corn. In another experiment, for example, growth of the main root went on in 1 per cent oxygen. Thus, 3 specimens were used November 5 and the roots grew 3.5, 1.5, and 3.5 mm. during the first day, and 1.5, 2, and 4 mm. the following day in this concentration. But the notation was made that the gas was streaming in at least one culture and in the other, with as rapid growth-rate, the tip of the root curved sharply. In continuing the experiment, helium was substituted for nitrogen, with the immediate effect that the rate of growth of all of the roots increased very considerably. Although, therefore, a certain amount of growth of the roots was demonstrated in 1 per cent oxygen in helium, and a slower rate in nitrogen, it should be noted that the rate was greatly below that expected in normal soil-air, and for the temperature which, at 22° C. for 24 hours, would be 22 mm. or over.

An experiment was started December 9 with 5 cultures of Early Mammoth field corn seedlings and at the same time and under the same cultural conditions, with one specimen of *Baccharis pilularis*, from the Carmel region, and one of *Fouquieria splendens*, from southern Arizona. Gaseous mixtures with 1, 1.2, and 2 per cent oxygen were used. The length of the roots of the corn was between 110 and 170 mm. at the beginning of the test.

The results for the first 3 days of the experiment and for corn, appears in table 19. Culture No. 5 is the control, with roots in normal soil-air. The growth of the roots in normal soil-air is given in the first column.

TABLE 19.—*Root-growth of Early Mammoth field corn, 1.2 per cent oxygen, with nitrogen*

Culture.	First day.		Second day.		Third day.	
	mm.	*° C.*	*mm.*	*° C.*	*mm.*	*° C.*
No. 1.....	48	24	1	24	0.5	24
No. 2.....	69	24	4.5	24	.5	24
No. 3.....	35	20	2.5	19	0	22
No. 4.....	..	..	2.5	19	0	22
No. 5.....	42	20	53	24	47	20

The sharp reduction in the amount of root-growth on the first day in the experimental atmosphere, and the total lack of growth the second day, indicate that 1.2 per cent oxygen is below the amount necessary for growth in the variety of corn used and for the temperatures. At the beginning of the second day in the mixture all of the cultures were irrigated, and during the second day the gas was passed slowly, but continuously, through them. But recovery was not effected. Then 2 per cent oxygen with nitrogen was given, but static, for 48 hours, with all of the cultures 23.5° C. There was no growth the first 24 hours in this gas, but during the second 24 hours two of the cultures increased 7.5 and 10 mm. in length, the balance not growing, neither did they recover later. Following the last, gaseous mixtures with 1 and 2 per cent oxygen were given alternately for 11 days, at two-day periods. As a result it can be said in general that recovery was made in the 2 per cent oxygen and that growth ceased in the 1 per cent, even when the gas was streaming.

It will be seen, therefore, that the results of the last series of experiments were somewhat at variance with the experiments with 1 per cent oxygen, reported in the earlier paragraph. The reason for this was not learned, although it was noted that during nearly the entire series the weather was cloudy and that in general plants did not appear to grow well. Immediately at the close of the experiments, therefore, and when there was a period

of sunshine, another series was started to determine whether there might be a relation between light and growth of the roots which might modify the rate of growth aside from the direct effect of the deficiency in the supply of oxygen. Four specimens were selected with roots from 130 to 200 mm. long; the temperature used was 23.5° C. Table 20 summarizes the reaction of the roots in 1.2 per cent oxygen, which is the same concentration as before.

TABLE 20.—*Root-growth of Early Mammoth field corn, 1.2 per cent oxygen, with nitrogen.*

Culture.	First day.	Second day.	Third day.	Fourth day.
	mm.	*mm.*	*mm.*	*mm.*
No. 6.	[1]49	6	4	0
No. 7.	38.5	Hid.	Hid.	Hid.
No. 8.	39	4.5	2	0
No. 9.	32	5	7	3

[1] The roots of all cultures were in normal soil-air the first day of the experiment.

In the continuation of the experiment, all of the cultures were aerated. Growth in 3 was immediately resumed and was between 15 and 32.5 mm. the first day in normal soil-air. No further growth, however, took place in No. 8.

It is interesting to note that in normal soil-air the root of No. 7, which it had not been possible to measure, as the tip remained hidden from view, came to the surface of the tube and grew 38.5 mm. in the 24 hours, as given.

The cultures were then given 0.8 per cent oxygen, in an attempt to repeat the earlier experiments in which 1 per cent had been used. As a result it can be said, in brief, that the root-tip of No. 6 turned away from the wall and was hidden from view, that No. 7 grew 2 mm. the first day and 1 mm. the second, and that No. 9 grew 5.5 mm. the first day, when the tip turned away from the glass wall.

All of the cultures were then aerated for 48 hours and in all a fair recovery was seen. Thus No. 6 increased 60 mm. during the time and No. 9, 80 mm.

Finally, 0.8 per cent oxygen was given once more, but the results were various and may have been in part due to the earlier treatment and need not be referred to further.

Although the experiments were not conclusive, they however did appear to point to a greater degree of toleration for an oxygen deficiency during the period of sunshine than during the immediately preceding period of cloudiness. The frequent loss of capacity for geotropic response, although the roots were still capable of a certain amount of growth, was also a marked characteristic of the reaction to so great a deficiency of oxygen.

Some additional tests were made with 0.2, 1, and 1.2 per cent oxygen. It can be said, briefly, that in 1.2 per cent for 48 hours 0.5 to 1 mm. growth took place in one plant and none in another, and that neither recovered subsequently, at a soil-temperature of 23.5° C. The experiment with 0.2 per cent oxygen is of interest only in that of 4 cultures, 3 had an added unknown amount of oxygen through the breaking of a distributing tube, when the roots grew, but no growth occurred in the fourth, and so as to the results with (0.8) 1 per cent oxygen, they were as before; that is to say, a decreasing rate took place from the first day. Taken altogether, therefore, the results appear to indicate that 1 per cent is near the lower critical concentration of oxygen for root-growth in the field corn for 23.5° C. In case other conditions favor, growth may go on at this partial pressure, but in case they are not favorable it does not do so.

Some experiments on Golden Bantam sweet corn were carried out with 3.6 per cent oxygen and at various soil-temperatures between 18° and 30° C. It will be sufficient to mention the results of the test, which was begun August 1, and which was continued for 5 days. 10 cultures were used whose roots were between 10 and 19.5 cm. in length when the plants were sealed in the tubes. Table 21 is a summary of the first part of the experiment.

TABLE 21.—*Root-growth, Golden Bantam sweet corn, in 3.6 per cent oxygen, with nitrogen.*

	Culture No.									
	1	2	3	4	5	6	7	8	9	10
Root length (cm.)	15.5	19.5	13.5	15	13.5	11	16.5	17.5	10	14.5
Soil temperature (° C).....	30	30	27	27	22	22	21	21	18	18
Growth, 24 hours, in normal soil-air (mm.).....	45	45	Hid.	30	22	11	32	28	Hid.	12
Growth, 24 hours, in 3.6 per cent oxygen (mm.)..	19	8	Hid.	5	Hid.	3.5	7	5.5	Hid.	8

It will appear from table 21 that a certain amount of growth took place in corn roots in 3.6 per cent oxygen at high as well as at low soil-temperatures and that at high temperatures it was fairly rapid. At the higher temperatures also there was a greater difference between growth in normal soil-air and in the gaseous mixture than at the lower temperatures. But in the experiment this was masked in large part by the hiding of the root-tips, and was only apparent in Nos. 1 and 10, in which the roots were in view on each day of the test.

At the conclusion of the test all of the cultures were aerated for 24 hours. This resulted in a normal renewal in Nos. 1, 6, and 10, but in all of the rest the tips of the roots were hidden from view. The cultures were then given 10 per cent oxygen for 24 hours with resulting normal growth-rate.

The experiment indicated, therefore, that 10 per cent oxygen may not be a deficiency at 30° C., or below, and suggests that it may not be so at higher soil-temperatures, although further experimentation would be necessary to definitely establish this.

In No. 2, which had the expected rate of growth initially in normal soil-air, there was seen to be a striking decline in the rate not only in 3.6 per cent oxygen, but subsequently, and not only during the first aeration referred to above and in 10 per cent oxygen, but in a subsequent aeration, not mentioned above. It was noted in this culture, but in none of the others, that numerous laterals were in process of forming between the beginning and the termination of the test. Thus the percentage of oxygen used did not obliterate the grand period of growth.

At the close of the experiment all of the cultures were carefully removed from the tubes and from the soil by jets of water, and were measured and otherwise studied. A very striking characteristic of all was the total lack of root-hairs, at least on the more recent growth. That this was not due to the process of washing away the soil was apparent from the fact that several other cultures, which were not used and which had not been sealed, were removed from the tubes at the same time, and in them root-hairs were abundantly present.

THE AERATION RELATIONS OF ROOTS: SOME GENERAL CONCLUSIONS.

The physiological activities of roots and the relation of such activities, directly and indirectly, to the environment are many-sided. Attention in the present investigation has been focussed of necessity on the relation of root-growth to the aeration conditions of the soil, although the student of roots early realizes that there are also important relations to other environmental features than aeration of the soil, and, in addition, that there are characteristics intimately associated with the root itself and independent of the environment which can not be lost to sight. To depict the root in its true relationship as an integral part of the "plant," or even to consider what may be the most important features of such relationship, granted that they are known, would be beyond the scope of this study. However, certain of the striking relationships of roots and of their reactions to differences in soil aeration, and something of the relation of such reactions to factors aside from the aeration of the soil and in addition to it, can be suggested in a few paragraphs.

In the development of the studies on root aeration it has become increasingly evident that the relation to the temperature of the medium in which the roots are placed is of very great significance. In fact, whether roots may continue growing in any given deficiency of oxygen may depend on temperature. Moreover, it appears that the cardinal temperatures for root-growth may be influenced by the oxygen-content of the substratum, or, to give the same idea other terms, by the rate of delivery of the oxygen to the root. Where the oxygen partial pressure is sub-"normal" there thus is an important relationship between temperature and the oxygen-supply. Now one may be the limiting factor in growth and now the other.

In addition to the features just mentioned is another of importance, namely, individuality of reaction. Both of these are encountered early by the experimenter. It is well known that in living organisms no two individuals, or even the same individual at different times or at different stages of development, are exactly the same. The possible reasons for such variability are sufficiently complex and varied and need not be referred to further in this place. The immediate and practical application of the matter is the necessity of studying each plant, as a separate individual, of learning certain of its reactions, and of utilizing this knowledge as an aid in interpreting results along any desired lines. Controls, in the usual sense of the term, are of only limited worth. In illustration of the necessity of observing so far as possible the reaction of the separate specimens used, some account will be presented of root variation under different circum-

stances. The illustrations will have to do mainly with the variation in growth in length of the primary root of seedlings and of related features.

TABLE 22.—*Root-growth in Prosopis velutina.*

No. of experiment.	No. of plant.	Temp. of soil.	Temp. of air.	Root-length at beginning of rapid growth.
		° *C.*	° *C.*	*mm.*
I	23	28.5	....	71.9
II	26	28.5	....	64
	27	28.5	....	60.7
	28	28.5	....	98
	29	28.5	....	90
III	31	30.5	21 to 24	118.4
	34	30.5	Do.	84.2
IV	41	34.2	....	52.2
	42	34.2	....	29.5
	44	34.2	....	34.5
V	45	31.5	22 to 24.5	63.2
	46	31.5	Do.	68
	47	31.5	Do.	52.5
	48	31.5	Do.	39.1
	49	31.5	Do.	38.3
VI	51	31.5	31 to 32.5	32.6
	52	31.5	Do.	18.2
	54	31.5	Do.	42
VII	51*a*	32 to 32.7	30 to 32	52.5
VIII	60	31.5	30 to 31	23.8
	61	31.5	Do.	31.7
	64	31.5	Do.	43.8

In table 22 is given a summary of observations on the length of the root of *Prosopis velutina* at the beginning of the period of most rapid growth, that is, the grand period of growth. The temperature of the soil is given, and in a few instances that of the air. The extremes are found in No. 52 and No. 31. In the former plant the root was 18.2 mm. in length and in the latter the root was 118.4 mm. in length at this time. The temperature of soil in both instances was nearly the same and was about optimal for growth of the root. However, the temperature to which the shoot was subject was in the plant last referred to about 10° C. lower than in the plant with the shorter root, but it is doubtful whether the observed difference is to be attributed to the difference in temperature of the shoot, for the reason that great variation was found where the temperatures of the shoot and of the root were alike and constant or not alike but yet constant. This will be learned by inspecting the table. The possible causes underlying the variation noted in the table, whatever they may have been, were thus not wholly due to temperature.

In certain species, especially in cotton, there appeared a relation between root-growth and the conditions of light to which the shoot

was subjected. It was sometimes observed, for example, that on dark and foggy days at Carmel, when the temperature of atmosphere outside of the culture cage was relatively low, the main root of cotton seedlings developed but poorly, although the temperature of the culture cage itself was favorable for root-growth. Under favorable conditions for root-growth the main root may become 12 cm., more or less, in length before laterals are formed, but under such conditions as those referred to laterals were formed while the tap-root was yet quite short, as, for example, 2 cm. long. It was observed that often in plants with so short a main root and so early development of laterals the laterals maintained an ascendancy for a relatively long time, how long was not learned. It is possible that such a beginning would shape the character of the mature root, as is known to be the case in other plants of longer life, which might be of exceedingly great importance from an ecological point of view.

The roots of all plants are subject indirectly to daily alternations in light and dark, and the rate of growth of the roots must be influenced by this fact, although to what extent or by what immediate agencies has not been determined. A few observations on seedlings of *Prosopis* were made in which growth of the roots was determined twice daily. Thirty-five day-night root-growth measurements were made and it was found that in 22 instances the root increased more during the hours of darkness than during those of light. Often, but not always, such roots were relatively young. The twice-daily growth variation for two successive days and nights is indicated in table 23. The 5 cultures were run at the same time, and in each instance the growth is from 6 p. m. to 6 a. m.

TABLE 23.—*Day-night root-growth in Prosopis velutina.*

No. of plant.	Length of root.	Temp.	Night.	Day.
		° *C.*		
26	38	28.5	11.8	14.5
			18.6	16.8
27	50	28.5	15.9	17.9
			20.2	20.1
28	72	28.5	15	17.3
			12.4	8.7
29	58	28.5	13.4	16.7
			19.1	15.8
30	58	28.5	11	12.7
			12.1	12.4

Although little appears to be known regarding the day-night variation in the stored-up food of roots, particularly the carbohydrate food reserve, it does seem unlikely that there may be such variation and that, if so, it might directly influence growth, inasmuch as with

high-carbohydrate reserve root-growth may be vigorous.[1] This would apparently be associated directly with the consumption of carbohydrates in darkness, with decreasing rate of respiration and with their reformation in light, when the rate of respiration increases.

Variation in root-growth is shown even more strikingly in the case of the main root of *Gossypium barbadense*, as indicated by table 24. Although the number of observations is too small to permit unquestioned conclusions, they do show, however, that the daily growth in the root of cotton is exceedingly variable, even at parallel temperatures of the soil.

TABLE 24.—*Growth of the main root of Gossypium barbadense, for 24-hour periods, and in normal soil-air.*

Temp. of soil.	No. of observations.	Daily growth.		
		Aver.	Max.	Min.
° *C.*		*mm.*	*mm.*	*mm.*
17.5	5	9.7	17.5	3.5
20.5	6	14	18	9
21.5	10	25.4	32	20.5
21.5	10	30.4	42	20
25	7	26	37	3
29.5	6	26	52	3
31.5	5	27.7	45	20
35	8	21	62	5

It should be stated as to the plants referred to in table 24, that the growth variation can not be attributed solely or even mainly to the grand period with the attending variation of growth. For example, in the case of two roots at the same temperature and observed at the same time, and with laterals just emerging in each, the root of one increased 52 mm. in length in 24 hours and that of the other but 16.5 during the same period; and, to cite another example, two plants were observed in which the roots were as yet without laterals. Both roots were at the same temperature of the soil. Of these, one increased 5 mm. and the other 8 mm. in 24 hours, while the roots of two other plants under similar conditions grew 13.5 and 3 mm. during a similar period.

Such experiences with root-growth as those summarized in table 24 indicate that although selected seedlings might react in a similar way to a common environmental complex, those taken at random do not do so. Such seedlings should be fairly representative. It does not seem to be warranted, therefore, to compare different plants, even of the same experiment, or merely to utilize controls. On the other hand, it is apparently feasible to relate, if possible, different plants

[1] Mary E. Reid, *Relation of kind of food reserves to regeneration in tomato plants*, Bot. Gaz., **76**: 193, 1924.

to a common standard having, if possible, none of the objections stated or implied by the foregoing remarks. This can be done by contrasting the growth-rate of the roots of any given plant while in "normal" or ordinary soil-air with the growth-rate of the same plant under conditions of the experiment. By "normal" aeration is here

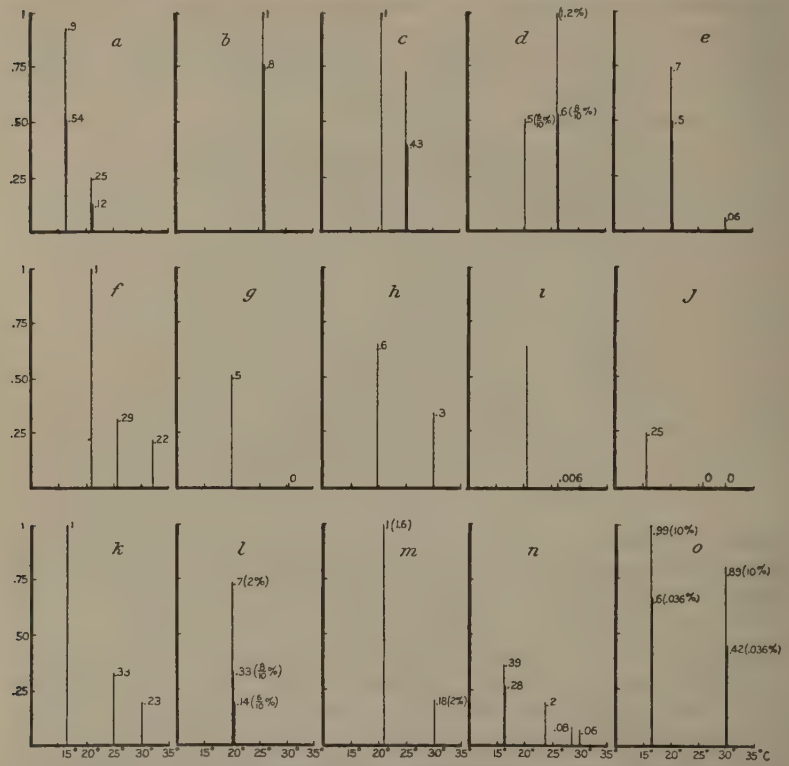

FIG. 19.—Growth ratio, r/R; relation of root-growth in normal soil-air (R) to the rate in experimental atmospheres (r). *Allium cepa*, *a*, 1.6 per cent oxygen; *b*, 2.8 to 3 per cent oxygen. *Citrus medica* var. *limon*, *c*, 2.8 per cent oxygen. *C. sinensis*, *d*, 0.6, 0.8, and 1.2 per cent oxygen. *Gossypium barbadense* var. *pima*, *e*, 2 to 2.6 per cent oxygen. *G. hirsutum* var. *acala*, *f*, 2.6 per cent oxygen. *Opuntia versicolor*, *g*, 1.2 per cent oxygen; *h*, 1.6 per cent oxygen. *Pisum sativum*, *i*, 1.6 per cent oxygen. *Potentilla anserina*, *j*, 1.2 per cent oxygen; *k*, 2 per cent oxygen. *Prosopis velutina*, *l*, 0.6, 0.8, 2.2, and 2.8 per cent oxygen. *Salix lasiolepis*, *m*, 1.6 and 2 per cent oxygen. *Zea mays*, *n*, 3 per cent oxygen; *o*, 3.6 and 10 per cent oxygen.

meant such as obtains in the culture tubes carrying the plants before the gaseous mixtures are administered. The expression is to be understood as conveying the idea that under such aeration conditions an increased oxygen-supply will not of itself necessarily bring about an increase in the rate of growth, the temperature and other environ-

mental conditions remaining constant. Moreover, such is apparently usually the case in nature, and where it is not an oxygen deficiency obtains. An oxygen deficiency, accordingly, occurs when, other conditions being equal, a subnormal rate of root-growth takes place in the partial pressure employed. It is convenient to express the growth-rate under "normal" and under experimental conditions by means of a ratio in which the growth-rate in the experiment (r) is divided by the "normal" rate (R), giving r/R, which can be conveniently expressed as a decimal. In such case 1 is the normal rate, or the rate previously determined to occur in the same plant before the

TABLE 25.—*Growth-ratios of roots at different temperatures and for different oxygen percentages.*

Species.	Growth ratio, r/R.	Temp.	Oxygen.
		° C.	*p. ct.*
Allium cepa	0.5 to 0.9	17 to 18	1.6
Do	.8 1	27	3
Citrus (lemon)	.5 .9	22	1.6
Do	.4 .7	27	2.8
Citrus (orange)	.5	22	0.6
Do	.6	27	0.8
Do	1.0	27	1.2
Opuntia versicolor	.5	20	1.2
Do	.6	20	1.6
Do	.3	30	1.6
Pisum sativum	.6	30	10.0
Potentilla anserina	.23	30	2
Prosopis velutina	.4	20	0.6
Do	.33	20	0.8
Do	.71	20	2.2
Salix lasiolepis	1.0	21	1.6
Do	.18	30	2.0
Zea mays	1.0	18	10.0
Do	.89	30	10.0
Do	.66	18	3.6
Do	.42	30	3.6
Do	.28 to .39	18	3.0
Do	.08	27	3.0
Do	.06	30	3.0
Do	.20	22	3.0

experimental gaseous mixture was given. The fractions so obtained can be plotted and studied quite as in case of actual growth measurements. As here suggested, it will be noted that the growth-ratio is for any given temperature and at any given concentration of oxygen. And it will be seen that although comparison may not be made between different plants as to the actual growth, such comparison as to growth-ratios is quite valid. The growth-ratio, therefore, permits direct comparison to be made between any desired stages in the development of the same species or individual. In table 25 are given tentative results showing the growth-ratio of several species, and at several different soil-temperatures, as determined in numerous experiments.

It has been shown above that in the daily rate of root-growth, with the roots at constant temperature and with normal soil aeration, there is not a little variation, the possible causes of which may not be apparent. The same remark would naturally apply to the growth-ratio. This can be illustrated by citing the growth of roots of *Gossypium barbadense* in subnormal concentrations of oxygen. The leading facts relative to growth of roots of the cotton are given elsewhere, to which the reader is referred for details. It is sufficient to state here that from 0.6 to 8 per cent oxygen, with nitrogen, was used, and at different but controlled temperatures of the soil. As a general result it was found that in 1 per cent oxygen, or less than 1 per cent, growth of the roots might or might not go on. In such low percentages of oxygen growth was especially doubtful at high temperatures. For the most part the results were negative, but as much as 6 to 10 mm. growth was recorded in a day in such oxygen concentration. In two instances the growth was 6 mm., giving a growth ratio of 0.1. In 1.4 per cent oxygen no root-growth was seen to take place at 30° or 35° C., although, as above suggested, it was occasionally seen to occur in somewhat lower concentrations at 30° C. Growth-ratios derived from using 1.4 per cent oxygen were as follows: at 16.5° it was 0.5; at 18° it was 0.33; at 20.5° it was 0.43. When the concentration was increased to about 1.7 per cent a growth-ratio of 0.8 was obtained at 20° and of 0.47 at 25° C. In 2.2 per cent oxygen, growth of the root appears to barely take place at 35°, and the ratio is correspondingly small. At temperatures between 20° and 26° C., the ratios are various. Thus, at 20° ratios between 0.67 and 0.8 were obtained, which, as will be seen, are not far below unity, signifying that 2.2 per cent oxygen at 20° is nearly a "normal" concentration for root-growth. At soil-temperatures between 23° and 26° C. the growth-ratios are relatively low. The ratio for 2.6 per cent oxygen was determined for *G. hirsutum*, but not for *G. barbadense*, to be 1 or normal at 21° C., and 0.29 and 0.22 at 28° and 32° C., respectively. In 4 per cent as well as in 8 per cent oxygen, the growth-ratio in *G. barbadense* does not exhibit so great variation up to about 30° as was found in the lower concentrations. This is probably owing to the relatively high concentration, which admits of a "normal" growth-rate for such partial pressures of oxygen and for temperatures below and up to 30° C. The soil temperature-oxygen partial pressure relation can be further illustrated by reference to root-growth in *Zea*. The following statement summarizes the leading results.

A partial pressure of oxygen equal to 3 or 3.6 per cent is a deficiency at all temperatures between 18° and 30° C. A normal rate of growth takes place in 10 per cent oxygen at 18°, but at 30° the growth-ratio is somewhat below 1 and indicates that for such soil-temperature 10 per cent oxygen is a deficiency. Moreover, it has apparently been the

experience of others that a partial pressure of oxygen, possibly equaling that of atmospheric air, may also constitute a deficiency in *Zea*. In such event, however, it must be assumed that the temperature of the soil or of the air was high, or that other factors not here taken into account importantly contributed to the result.

In the preceding paragraph it was shown that the growth-ratio could be modified by changing the temperature of the soil, provided the concentration of oxygen was not sufficient to bring about a normal rate of growth. It was also remarked that, in event of an oxygen deficiency, the ratio of growth would also be changed if the partial pressure of oxygen was increased or decreased. This was observed in several instances. Thus, at 20° C. in *Prosopis* a ratio of 0.33 to 0.4 was obtained with 0.6 to 0.8 per cent oxygen, and a ratio of 0.7 with 2.2 per cent oxygen, at 20° C. in both cases. To mention only one more example, in the orange, at 27° C., the ratios were 0.6 and 1 in 0.8 and 1.2 per cent oxygen, respectively.

The instances in which the growth-ratio in roots was seen to be changed by change of temperature, or by modifying the oxygen-content, always in event of an oxygen deficiency, might be extended. But enough has been given to indicate the general nature of the relation. It will not fail being noted that, as repeatedly stated, the relation has to do with an oxygen deficiency. In case there is no deficiency an increase in the concentration of oxygen should not of itself bring an increase in the rate of growth, whatever the temperature may be.

From what has been presented above it will be seen that there are two or more important features in the conception of the relation of root-growth to a deficiency of oxygen and to temperature. Of these, one has to do with the least concentration of oxygen at which root-growth will continue at any temperature of the substratum, the lower critical concentration, and a second has to do with such oxygen-supply as will just permit a "normal" rate of root-growth for any temperature. This is the upper critical concentration. As to the last it can be explained that by a "normal" rate of root-growth for any temperature is meant in short such a rate as goes on in a soil-atmosphere which is "well" aerated. In other words, a rate of growth is "normal" when an increase in the oxygen partial pressure may not result in an increase in the growth-rate, the temperature and other conditions being constant and equal. Again, a "normal" rate of growth of roots, for any temperature of the soil, takes place only in optimal concentration of oxygen in the substratum. Below this is an oxygen deficiency; above this increase in temperature induces a slowing of the rate of growth. There thus may be said to be four cardinal concentrations of oxygen for root-growth. The lower critical or least concentration consistent with growth, and the upper critical or the least concentration at which a normal rate of growth will take

place, have already been mentioned. Between these there exists an oxygen deficiency. The upper critical could also be termed the lower optimal concentration. With concentrations above the upper critical the present study is not concerned, but it does not appear improbable that the range of the optimal concentration, including its upper limits, as well as the maximum concentration for root-growth, may either or both be characteristic of a species, just as the lower critical and upper critical concentration as well are all apparently characteristic of species or of types of plants.

An additional word should be said relative to the range of the lower critical and upper critical concentration of oxygen and that of oxygen deficiency. To determine these surely would require more experimentation than has been possible to devote to the matter. However, certain features can be pointed out and possibly the general relation can be established with the data at hand. The range of the upper critical concentration will be spoken of in the first place. Taking *Zea* for an example, it will be seen that the growth-rate is nearly normal, with the growth-ratio about 1, at a temperature of 30° C. and when the oxygen concentration equals about 10 per cent. Below such temperature, therefore, the rate of growth would vary directly with variation in the temperature of the substratum, other factors being equal. It is not likely that a normal rate would be had in this concentration were the temperature increased to 35° C., or any temperature between 35° and 30° C., but for the purpose of illustration it will be assumed that a normal growth-rate will go on in *Zea* at 35° C., and in 10 per cent oxygen. At a soil-temperature of 18° C. and 3.6 per cent oxygen, the growth-ratio is about 0.66. What the concentration of oxygen would be to effect a normal rate of growth at 18° C. was not determined, but it will be assumed to be about 4 per cent. The growth-ratio in less than 4 per cent, at 18° C., therefore, would be less than 1. The range of the upper critical concentration of oxygen as between 18° and 30° C. for *Zea*, is, therefore, about 6 per cent. From this it will appear that the range of the upper critical concentration of oxygen for *Zea* is relatively great.

What the range of the lower critical concentration of oxygen for root-growth in *Zea* may be was also not determined exactly, but it may possibly be indicated by certain observations. It was doubtfully shown that root-growth in *Zea* goes on at any temperature in 1 or 1.2 per cent oxygen, and growth of the roots of *Zea* is nearly *nil* at 30° C. if the oxygen concentration equals 3 per cent. Although further studies might have modified these percentages somewhat, it appears probable that they are not far wrong. It may be, therefore, that the range of the lower critical concentration of oxygen, between 18° and 30° C., is about 2 per cent in *Zea*.

The range of oxygen deficiency is limited by the upper critical

partial pressure for the highest temperature for root-growth, on the one hand, and on the other by the lower critical concentration at the lowest temperature for root-growth in the species. In *Zea* the extreme range of oxygen deficiency, therefore, is not far from 9 per cent, and may be greater than this.

Although as before-mentioned opportunity was not afforded to determine the critical oxygen concentrations for many species, a glance at table 25 will indicate that the concentration of oxygen is unlike for plants which tolerate marked oxygen deficiency from what it is in plants, such as *Zea* and others, which are not capable of doing so. Thus, in the orange, at 22° C., root-growth appears to be relatively active in about 0.6 per cent oxygen, and at 27° C. is about normal in 1.2 per cent. It is clear, therefore, that in this species the range of the lower critical and the upper critical concentrations, as well as that of oxygen deficiency, is strikingly small. The lemon apparently has a higher upper critical concentration than the orange for 27° C. and possibly for 22° C. as well. The data do not give the range of the critical percentages in *Salix*, but they suggest that while the species can maintain a normal rate of growth at 21° C. in 1.6 per cent oxygen, more than 2 per cent is required for normal growth when the temperature is raised to 30° C. The species, therefore, is far from being able to continue root-growth in the absence of oxygen in the soil.

The general principles relative to oxygen partial pressure, temperature of the soil, and root-growth can be somewhat further substantiated by especial reference to studies carried out on *Gossypium barbadense*, certain of which have already been mentioned. Table 26 summarizes the leading results. In the cotton the soil temperatures most favorable for growth lie between 22° on the one hand and 30° or 32° C. on the other. Below 22° and above 32° the rate of growth, under normal conditions of soil aeration, falls away rapidly. As the table indicates, the concentrations of oxygen used were between 0.6 and 8 per cent, and the temperatures employed were between 17° and 35° C. In 0.6 per cent oxygen growth doubtfully occurs at 17° C. and is very slow at the most favorable temperatures for growth, but in 8 per cent oxygen root-growth takes place at all of the temperatures used and is about normal at 30° and below. The lower critical concentration of oxygen at 17° C. for growth of roots in the cotton is therefore about 0.6 per cent or more, and the upper critical is 8 per cent or more. At the higher temperatures the lower critical partial pressure is about 2.4 per cent, giving thus a range of the lower critical of about 1.8 per cent. Although the minimum upper critical concentration was not definitely established, it appeared to be not far from 2 per cent. The entire range of this concentration, therefore, was found to be about 6 per cent.

The range of the oxygen deficiency in cotton will be seen to be

nearly, if not quite, as great as was found to be the case in *Zea*. It is not far from 7.5 per cent.

TABLE 26.—*Root-growth ratios of Gossypium barbadense, at different temperatures and for different percentages of oxygen.*

Oxygen.	Temp.	Growth-ratio, r/R.		
		Aver.	Max.	Min.
p. ct.	°C.			
0.6 to 1	18 to 20	0.012	0.05	0
	21 25	.10	.25	0
	26 30	.145	.19	.10
1.1 1.4	18 20	.425	.50	.33
	21 25	.70	....	....
	25 30	.09	.9	0
	31 35	0	0	0
1.5 2	16 20	.296	.16	.80
	21 25	.467	1.00	.25
	26 30	.30	.41	.27
	31 35	0	0	0
2.1 2.4	16 20	.498	.70	.08
	21 25	.806	1.00	.38
	26 30	.337	.45	.18
	31 35	.02	.03	.01
2.5 3	17 20	.37	....	....
	21 25	.96	....	....
	26 30	.44	.57	.31
	30 35	....	....	....
3.1 8	19	.54	....	....
	21 25	.993	1.00	.96
	26 30	.78	1.00	.38
	31 35	.62	.86	.38

The cardinal temperatures for root-growth appear to undergo marked change in case of a deficiency of oxygen, as might be expected. This can be illustrated by reference also to the results with the cotton. Under normal conditions of soil aeration the maximum temperature for root-growth in *Gossypium* is above 35° and the optimal temperature is not far from 25° C., as has already been mentioned. In case the amount of oxygen in the soil is reduced to about 2.4 per cent when at the same time the temperature of the soil becomes about 35° C., or possibly less, growth of the roots ceases. Similarly, the optimal temperature for root-growth may be depressed in a deficiency of oxygen. Thus, in 2 per cent oxygen, the optimal temperature may be 4° or more below the "optimum" for root-growth in the species. Whether the minimum temperature for growth can be modified a change in the concentration of oxygen was not observed. In extreme oxygen deficiency there may possibly be but one "cardinal" temperature for growth, namely, the minimum. It seems, therefore, that the temperature at which root-growth is most rapid and at which it ceases because it is high should be defined in terms of the amount of oxygen available for the root.

The relation between root-growth and the normal or optimal oxygen concentration, on the one hand, and to subnormal concentration of oxygen or oxygen deficiency, on the other, in relation to various soil-temperatures, is shown graphically in figure 20. The figure is based on observations on *Gossypium*, but it is applicable in principle to all of the species studied in this regard.

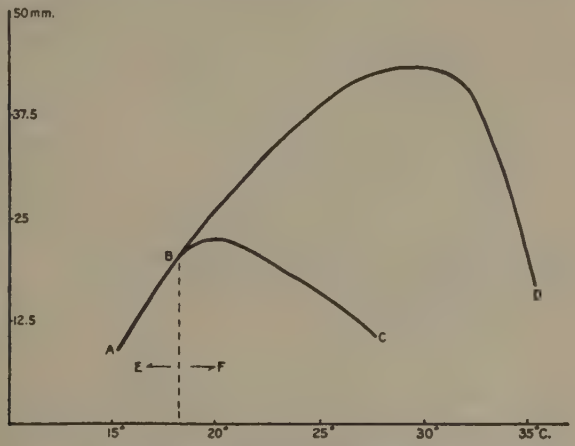

FIG. 20.—Oxygen deficiency *F* and a sufficient oxygen supply *E* as related to root-growth at different soil temperatures. Semi-diagrammatic, based on *Gossypium barbadense*. *ABD*, daily growth in normal soil-atmosphere. *ABC*, daily growth in approximately 2.2 per cent oxygen. Note that the optimal and the maximal temperatures for root-growth are much lower in the smaller oxygen-supply.

The curve *ABD* is a rate curve of growth under "normal" conditions of soil-aeration, for 24-hour periods, and at 15° to 35° C. The curve *ABC* is growth in about 2 per cent oxygen and at 15° to 30° C. At about 20° and below the growth-ratio is 1, so that in the coincident portion of the curves, *A–B*, temperature and not the supply of oxygen is the limiting factor. Below about 20° C., therefore, there is a sufficient supply of oxygen, and an increase in the concentration may not induce an increased rate of growth. It will also be noted that the optimum temperature for growth of the root subjected to such oxygen deficiency, 2 per cent, is about 20° C. Greatly above this temperature an increase in the temperature is accompanied by a decrease in the growth-rate, with the final effect that at about 30° growth ceases. Between 20° and 30° C., therefore, the growth-ratio is less than 1 and there is an oxygen deficiency. The oxygen concentration in such temperature range is the limiting factor of root-growth.

Figure 20 is based on root-growth in about 2 per cent oxygen. If a curve were constructed for a greater concentration of oxygen, the

general form would remain the same, but the optimum temperature for growth would be at a higher temperature than 20°, and, conversely, a curve of growth in less than 2 per cent oxygen would show a depression of the optimal as well as of the maximal temperature for root-growth, as already explained in an earlier paragraph

Figure 20 has to do with actual growth measurements, but it is evident that the curve *AC*, representing root-growth in about 2 per cent oxygen, can as well stand for growth-ratios. Under such circumstances in the coincident portion, *AB*, the growth-ratio would

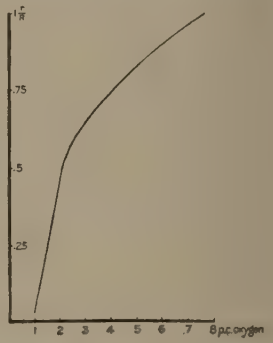

FIG. 21.—Growth-ratio (r/R) in *Gossypium barbadense*, constant temperature 30° C. in a soil-atmosphere containing from 1 to 8 per cent oxygen.

be 1, but in the balance of the curve it would be less than unity. The relation of any portion of *BC* to the corresponding portion of *BD*, which would be the growth-ratio, could be learned with little difficulty, and if plotted would assume the form of the figure. Such a curve is for a given concentration of oxygen.

When the growth-ratio for any species is represented in a graph where the temperature is constant, as in figure 21, other characteristics appear, certain of which have already been noted. In the figure, which is based on experiments with *Gossypium barbadense*, an approximation is presented of root-growth in different percentages of oxygen and at a soil-temperature of 30° C. It will be seen that except for the highest concentrations the graph represents root-growth in an oxygen deficiency only. In the first portion of the curve, representing growth in about 2 per cent oxygen, the rise of the growth-ratio is exceedingly rapid, but in the balance of the curve the rise is continuously less rapid, flattening out at the approach of the upper critical concentration and the optimal partial pressure. It appears possible that unlike forces are in operation to determine the leading characteristics of the two portions of the curve. The steep portion may be more closely associated with the lower critical concentration and the upper and more flattened portion with the upper critical concentration of oxygen, although it would require graphs for several

temperatures to determine this. At lower temperatures more of the curve would be flattened and at higher temperatures more would be steep. The figure is for a species not tolerant of a poor oxygen-supply. In such plants as are not so closely tied to a good supply of oxygen, as is the case of cotton, the steep portion of the curve would be relatively short, but the leading character of the curve would possibly otherwise remain the same.

It has been shown that in all of the species studied in this particular the range of the lower critical concentration of oxygen in any plant has been found to be small, and thus not to be very different as between species with unlike oxygen relations. This range appears to nearly parallel the amount of oxygen which water is capable of absorbing for the same temperatures. As is known, the solubility of a gas in a liquid is directly affected by several factors, among which are to be included temperature, the partial pressure of the gas, and the density of the solution. The solubility of the gas varies inversely with the temperature and density of the solvent and directly with the partial pressure of the gas.[1] As to variation of solubility in solutions of different densities, the authors referred to state, for example, that at 760 mm. Hg pressure and at 15° C., the coefficient of solubility of oxygen varies from 0.033 in 0.5-gram equivalent solution to 0.029 in 2-gram equivalent solution of HNO_3, and from 0.029 to 0.017 in corresponding densities of NaCl. While these remarks may apply to the soil solution, they apparently do not necessarily apply to the plant solution where various physiological processes and conditions may enter to modify or reverse the relation, as stated above.

TABLE 27.—*Amount of oxygen, by weight, dissolved in 1 liter water, 760 mm. Hg pressure, at various temperatures. (1.42 grams* $\times T/T+t$*).*

T.	Grams
° *C.*	
15	1.34
20	1.32
25	1.30
30	1.27
35	1.25

TABLE 28.—*Coefficient of absorption of oxygen in water (A), volumetric relations, 760 mm. Hg pressure.*

T.	A.
° *C.*	
15	0.03415
20	.03102
25	.02831
30	.02608
35	.02440

In tables 27 to 29 are given quantitative data relative to the absorption of oxygen and atmospheric air by water at the temperatures employed in the experiments. Useful mainly for illustration, they show, however, the important fact that a larger amount of oxygen is available at the lower soil-temperatures than is available at temperatures which are relatively high.

[1] Landolt, Börnstein, and Roth, Tabellen, 5 Aufl, 1912.

The volumetric relations of oxygen absorption in water, under standard conditions of pressure, and for the temperatures mostly used in the experiments, are given in table 28.

The same authors give also the solubility of air, together with its most important component gases, in water. Table 29 reproduces this data in part. It will be seen that oxygen constitutes about one-third of the dissolved air, so that the proportion of oxygen in water, as opposed to the proportion in atmospheric air, is relatively large. Referring now to the concentrations used in the present experiments, it will appear that when the gaseous mixtures contained 1 per cent

TABLE 29.—*Amount of air dissolved in 1 liter water.*

T.	Total.	Oxygen.	Nitrogen, etc.
° *C.*	*c. c.*	*c. c.*	*c .c.*
15	20.55	7.04	13.51
20	18.68	6.36	12.32
25	17.08	5.78	11.30
30	15.64	5.26	10.38

oxygen, which was found to be about the lower critical concentration for root-growth in cotton and some other species, the soil solution contained approximately 0.07 c. c. oxygen per liter at 15°, and approximately 0.056 c. c. oxygen per liter at 30° C.; and at about the upper critical concentration of oxygen the soil solution contained approximately 0.56 c. c. oxygen at 15° and 0.4 c. c. at 30° C. At 35° C. the amount of oxygen in solution would be less, but the amount is not given in the work cited.

TABLE 30.—*Approximate oxygen content of soil solution, with percentages of oxygen, at lower and upper critical concentrations of oxygen, for cotton.*

Temperature.	Lower critical concentration.		Upper critical concentration.	
	Oxygen.	Oxygen per liter.	Oxygen.	Oxygen per liter.
° *C.*	*p. ct.*	*c. c.*	*p. ct.*	*c. c.*
17 (15°)	1	0.07	2	0.14
30	2.4	.12	8	.42

The solubility tables give an approximation of the quantitative oxygen relations in root aeration. It will be noted that the tables have to do with the oxygen-content of water-masses and not with water-films. Further, they deal with pure water. In regard to the former, it is evident that the forces concerned with the absorption of oxygen by water-films, such as occur in well-aerated soils for example, are widely different from the forces operating in water-masses. Owing to differences which are possibly mostly associated with variation in surface tension, a greater amount of oxygen is probably absorbed by

films than by masses of water, and a correspondingly larger amount of oxygen is available to the root in parallel partial pressures of that gas. The difference in the oxygen relations of plants in well-aerated and poorly-aerated soils, therefore, may be even more striking than the differences in the concentration of oxygen contiguous to the water-film or water-mass may suggest. What the difference may be appears not to be known, so that it will be necessary, for the present purpose, to assume that the oxygen-content of the two classes, water-films and water-masses, is essentially alike. Referring to table 30, it will be seen in relation to the lower critical concentration, and for cotton, that there is a rough parallel between the increased oxygen requirement accompanying the higher temperature and the oxygen-absorption capacity of water for the same temperatures. The oxygen-content of water decreases with increase in the temperature, so that a corresponding increase in the partial pressure of the oxygen in the atmosphere is required to maintain an even concentration when the temperature is raised. The actual increase in oxygen-concentration is about 170 per cent as indicated experimentally for the lower critical concentration, and about 132 per cent as indicated by solubility tables.

Table 30 also indicates the relatively large range in the magnitude of the upper critical concentration of oxygen in the cotton. As determined experimentally, the increase in concentration of oxygen from 15° to 30° for the upper critical is approximately 400 per cent, but as indicated by the solubility tables it is only 300 per cent. The range of the upper critical concentration in species which tolerate marked oxygen deficiency will possibly be found to more nearly parallel the differences in absorption capacity of water for the same temperatures.

In the total absence of researches covering the subject, it would be futile to suggest possible reasons underlying such a fundamental difference in the range of the magnitude as that above shown to lie between the lower and the upper critical concentrations of oxygen in relation to the growth of roots. It appears, however, as mentioned before, that the range of the lower critical concentration is not far different in all species investigated in this regard. As a criterion on which to base the relative aerobism of species this is clearly not wholly satisfactory. On the other hand, it has been shown that the upper critical concentration may vary greatly as between different species, being especially accentuated in this regard in markedly aerobic forms. It would appear possible, therefore, to associate such variation with determined oxygen-content of the substratum, and in this connection it is of interest, at least, to note that the oxygen-content of different soils as now known is of such magnitude as to come within the upper critical concentration for root-growth in plants. This can be suffi-

ciently illustrated by reference to the findings of Leather in Indian soils.[1]

It is hardly necessary to point out that the relation of root-growth to the oxygen-supply can not be inferred only by the behavior of roots as observed in the field, nor can practical application of laboratory results be safely made by inspection of the soil. The two should be related, so far as possible. The oxygen-content of the especial soil should be told, or at least the rate of oxygen delivery should be known, and this should be directly connected with the known oxygen-content or rate of delivery in the given experiments. Further than this the temperature of the substratum should be known. The importance of the latter was not appreciated in the earlier work on aeration and root-growth. But it appears that species which may usually be considered as capable of maintaining growth in extremely small concentrations of oxygen may nevertheless not do so in event of high soil-temperature, and, conversely, that species which may require a good oxygen supply may continue root-growth in small oxygen concentrations, provided the temperature is low. It is possible, also, that the stage of the development of the shoot, or factors directly influencing various activities of the shoot, or both, may indirectly influence root-growth, and importantly so, in event of critical concentrations of oxygen. But of this little or nothing is exactly known.

GENERAL SUMMARY OF SECTION.

Effect on Root-Growth of Excessive Concentration of Carbon Dioxid in the Soil-Atmosphere.

(1) Root-growth in *Covillea tridentata* ceases promptly in a soil-atmosphere containing 25 to 75 per cent carbon dioxid, and growth may or may not be renewed when the gaseous mixture is replaced by atmospheric air.

(2) The roots of *Mesembryanthemum aequilaterale* are fairly resistant to carbon dioxid in concentrations between 25 and 75 per cent. Although growth ceases after relatively long exposure to a gaseous mixture so rich in carbon dioxid, it is renewed relatively soon upon aeration of the soil.

(3) When the root of *Krameria canescens* is exposed to 25 per cent carbon dioxid, or even to a higher concentration, provided the temperature of the soil is about 30° C., growth ceases, but if the soil-tempera-

[1] J. W. Leather, *Soil gases*, Mem. Dept. Agr. India, Chem. Ser. 4: 85. In unmanured lands, in spring, Leather found from 13.5 to 18.6 per cent oxygen at various depths up to 5 feet. In October, and in manured land, there was from 11.4 to 17.8 per cent oxygen, but in swamp land he found only 0.54 per cent. Some determinations were also made of the oxygen-content of the soil immediately about the roots of *Crotolaria*, *Indigofera*, and *Zea*. In the first, at a depth up to 15 inches, there was from 2.23 to 9 per cent oxygen in the second species from 3.33 to 5.24 per cent, and in the third species from 6.28 to 13.82 per cent.

ture is about 20° C. the root may continue to grow for a relatively long time in a mixture containing 75 per cent.

In paragraphs 1 to 3 is given summary of results of experiments in which the concentration of oxygen was less than "normal." The succeeding paragraphs give results of experiments conducted with an excess of carbon dioxid but with a "normal" partial pressure of oxygen.

(4) Roots of *Prosopis velutina* in 25 per cent carbon dioxid and 20 per cent oxygen continued to grow 7 days, although the rate was approximately 30 per cent less than the rate would have been in normal soil-atmosphere. In a gaseous mixture containing equal parts of carbon dioxid and oxygen a slow rate of growth continued for 72 hours. In 75 per cent carbon dioxid with oxygen, a momentary growth acceleration resulted, followed by a gradually decreased growth-rate.

(5) The roots of cuttings of *Opuntia versicolor* were exposed to 75 per cent carbon dioxid and 25 per cent oxygen; 25 per cent carbon dioxid and 75 per cent oxygen; and equal parts of carbon dioxid and oxygen. The experiments with an excess of oxygen gave negative results. In 75 per cent carbon dioxid root-growth promptly ceased, but with the admission of atmospheric air it was quickly resumed.

(6) Roots of *Krameria canescens* were given 25 per cent carbon dioxid with 25 per cent oxygen, and 25 per cent carbon dioxid with 75 per cent oxygen. In the mixture containing an excess of oxygen a brief acceleration of growth took place, followed by a decrease in rate. Only a depressing effect on growth was observed in the gaseous mixture containing 25 per cent each of carbon dioxid and oxygen.

The succeeding paragraphs give results of experiments on *Opuntia versicolor* and *Prosopis velutina* with roots in varying concentrations of carbon dioxid and with or without normal oxygen partial pressure.

(7) The root of *Opuntia* in a soil-atmosphere containing 50 per cent carbon dioxid usually continued growing, although at a subnormal rate, for 3 hours. Recovery took place about 3 hours after aeration.

(8) When the roots of *Opuntia* were exposed 3 hours to 75 per cent carbon dioxid the growth-rate was greatly decreased, but growth did not usually stop. Recovery took place sooner at temperatures about optimum for growth than at temperatures about 10° C. below this.

(9) At the optimum temperature for root-growth an experimental atmosphere containing 90 per cent carbon dioxid did not cause growth to wholly cease. Recovery occurred from 3 to 6 hours following aeration.

(10) Cylinder carbon dioxid was used in several experiments on *Opuntia*. In the experiments the length of exposure, the length of time necessary for recovery from the effects of the gas, and, in some instances, the relation to temperature were observed.

At relatively low soil-temperatures, 30-minute exposures, growth

usually stopped during the gas administration, and recovery did not take place until 2 or 3 hours after atmospheric air had been run into the culture At about optimum temperature, however, the rate of growth was greatly lowered, but growth did not wholly cease. Recovery took place during the first hour following aeration.

When given the gas 60 minutes, slight growth took place early in the gas administration. No growth was observed for 2 hours following the admission of air, but recovery occurred about 3 or 4 hours after the cultures were aerated. An exposure for 2 hours had about the same result.

Roots of *Opuntia* which were given cylinder carbon dioxid 3 hours and were then aerated were quiescent for about 6 hours, but recovery was finally made. The recovery was related to the temperature, being sooner at about optimum for root-growth than at about 10° C. below.

When 50 per cent carbon dioxid was used the rate of growth was seen to be greatly depressed, and if the exposure-time was prolonged growth ceased. The inhibitory effect was seen to continue for about an hour after the cultures had been aerated. Temperature effects were also noted as before.

Root-growth and the Oxygen-Supply.

(11) The reaction of roots to a deficiency of oxygen was observed in about 30 species, varying greatly in habit and often from unlike habitats.

(12) When oxygen was entirely removed from the soil in which the roots were growing growth ceased in all species.

(13) Many of the species, possibly most of them, were found to maintain a certain although slow growth-rate in as little as 0.5 per cent oxygen for a limited period of time.

(14) Preliminary experiments suggest the possibility that in event of a very low partial pressure of oxygen in the soil there may be a slow downward movement of oxygen into the root from the shoot, and finally into the soil from the root itself.

(15) When the partial pressure of oxygen was too low to permit a normal rate of growth, the growth-rate was found to vary inversely with the temperature, being more rapid at low temperatures and less rapid at temperatures which were high.

(16) At a constant temperature and in low partial pressures of oxygen, the rate of growth was found to vary directly with the partial pressure of that gas.

(17) For all species studied there was found to be a concentration of oxygen in which growth would not continue, whatever the temperature may have been. For convenience, the lowest concentration of oxygen at which growth will just take place for any temperature is called the lower critical concentration for the species and for the tem-

perature. The lower critical oxygen concentration, in all of the species examined, was greatest at the highest temperatures and least at the lowest temperatures.

(18) The extreme differences in the lower critical concentration of oxygen for growth *as between the species* studied did not appear to be over 1 or 2 per cent, at about corresponding temperatures, and the range of the lower critical concentration *for any species* did not appear to exceed 1 per cent as a rule.

(19) The range of the lower critical concentration of oxygen between the extremes of growing temperature for roots, and for any one species, about equals the differences in the oxygen-absorption capacity of water for the same temperatures.

(20) A second cardinal oxygen concentration for root-growth is such a partial pressure as will just permit a "normal" rate of growth for any given temperature. With the temperature constant, an increase in concentration above such concentration will not induce an increase in the rate of growth. This is here referred to as the upper critical concentration of oxygen. It can also be termed the lower optimal partial pressure, and has a characteristic similar to the lower critical partial pressure for growth, in that its value is inversely related to temperature. The least upper critical concentration occurs at low temperatures and the greatest at high temperatures, but the range of the upper critical, for any species, may be from 2 to 8 per cent, more or less, and, thus is much greater than the range of the lower critical concentration.

(21) The difference between the lower and the upper critical oxygen concentration for root-growth is the range of the oxygen deficiency. This range is apparently specific and is directly related to the degree of aerobism of a species, being greatest in those most dependent on a good oxygen-supply.

(22) Although species may and do differ as regards the relation to the lower critical partial pressure of oxygen, it is possible that the most striking specific differences in the oxygen relations have to do with the upper critical concentration and its variation in association with temperature.

(23) The cardinal temperatures for root-growth may be directly modified by the concentration of oxygen. In an oxygen deficiency both the maximal and the optimal temperatures for growth of roots are lowered, but the minimal temperature was not noted to be changed from such cause.

(24) It is the rate of supply rather than the partial pressure *per se* of oxygen that influences the rate of growth. Thus, in species where the oxygen concentration was below the lower critical concentration and growth of the root would not continue with the experimental atmosphere static, it would begin if the gaseous mixture were caused

to pass slowly through the soil. Further, as shown in the preceding sectional summary, in parallel percentages of oxygen a mixture of nitrogen with oxygen is less efficient in influencing the rate of root-growth than a mixture of oxygen with helium.

(25) Occasional observations on the formation of root-hairs indicate that in certain species of marked aerobism root-hairs do not develop in event of great oxygen deficiency, as for example, about the lower critical concentration.

(26) Species which require a relatively high percentage of oxygen for root-growth failed to react positively to gravity in a low concentration of oxygen. Thus in such species growth continued at a partial pressure of oxygen below that required for the geotropic reaction. Whether such would also be the case in species not so markedly aerobic was not determined.

(27) In *Prosopis* and in other species, seedlings which were seen to be most vigorous appeared to be most resistant to a deficiency of oxygen.

(28) There appeared to be a direct relation between the size of the cotyledons in *Prosopis* and the oxygen-supply. The cotyledons are relatively small in a low-oxygen partial pressure.

(29) The hypocotyls of *Prosopis* appear to be insensible to deprivation of oxygen.

(30) The first observable morphological effect of an insufficient oxygen-supply in *Pisum* was seen to consist of a shrinking of the meristematic tissue of the root-tip. It is evident that the loss of turgor is directly induced by the failure to absorb water as a direct effect of the anaerobic condition obtaining. If such condition proceeds beyond a certain stage, recovery with the admission of atmospheric air does not take place; if, however, the reaction has not progressed far, recovery may be possible. In the last event the effects of the reaction to a low concentration of oxygen may persist for a relatively long time as a constriction of the root.

(31) In some instances the growth of a root in a given percentage of oxygen and at a given temperature is expressed in relation to growth at the same temperature but under optimal conditions of aeration. This is the growth-index or growth-ratio or relative growth of the root of the species and for the temperature and partial pressure of oxygen. It can be expressed by the fraction r/R and can be plotted in the same way as actual growth. By the use of relative growth, unlike species can be compared directly as to the degree of aerobism with relation to temperature and oxygen concentration.

(32) Mention should be made that oxygen may diffuse slowly into a sealed culture, although the seals may be sufficiently tight to prevent molar gas movements. This was found to be the case, with low partial pressure of oxygen in the soil, in several species, including cotton, corn,

Potentilla, and onion. In bulbous forms, however, the inward diffusion was apparently between the scales, and also takes place where the leaf-bases are closely pressed together, as in *Potentilla*. In such species sealing at the crown of the main root seemed to prevent the inward movement largely, or wholly. However, in many instances, as in cotton, where the leaves were not crowded at the base of the stem, there were indications of inward diffusion of oxygen. The conclusion is given with reservation, but in many species it was observed to have occurred. In preliminary experiments looking to the further examination of the matter, it was learned that in darkness controls and cultures behaved in a similar way, but very often a greater percentage of oxygen was demonstrated in the culture tubes in plants which had been illuminated.

(33) A comparison of results indicates that there may often be a close relation between the degree of aerobism of a species and the occurrence of the species in a given habitat, although it has not been possible, for want of data on the oxygen-content of the soil, to make the comparison with exactness. The observed possible relation can be succinctly given as follows: Such species as *Baccharus viminea*, *Citrus sinensis*, *Juncus effusus* var. *brunneus*, *Potentilla anserina*, and *Salix lasiolepis*, which occur where the substratum may be saturated a part of the year, are able to maintain a certain rate of root-growth in a soil-atmosphere containing 0.5 per cent oxygen. No species which requires a relatively high percentage of oxygen for root-growth has been found to occur in such moist habitats. But species in a habitat with well-aerated soil, in contrast to the above, may have unlike relations to the oxygen-supply. Thus, at Carmel, where the soil is sand, are to be found *Mesembryanthemum* and *Eriogonum*, both of which are relatively but unequally insensible to oxygen deprivation, and also *Stachys*, which, on the other hand, apparently requires a good oxygen-supply for root-growth. The soil of this habitat is never puddled. *Covillea*, *Fouquieria*, and *Opuntia*, all of which are relatively intolerant of poor conditions of soil aeration, grow on the bajada slopes near Tucson. The soil of the bajada is coarse and probably well-aerated most of the year, but during the rainy seasons it is frequently puddled. Even so, however, where water seepage occurs there may be translocation of oxygen in solution in sufficient amount to permit root-growth and water absorption by the plant, even if direct gaseous exchange by diffusion between the atmosphere of the soil and that of the air under such conditions is slow. The species last mentioned are largely absent on the flood-plain, where the soil is adobe which is saturated during the period of high temperature and where lateral water-movement is not active. Among the species indigenous to the flood-plain in the immediate vicinity of Tucson is *Prosopis*, which is relatively insensible to oxygen deprivation, and,

among the cultivated species, alfalfa, which also appears not to require good aeration for root-growth.

(34) In the matter of the direction of root-development, the branching of roots, or the like, the experiments have little to offer in the way of explanation. Uncontrolled experiments with *Opuntia* indicated that branching of the roots was most abundant in those portions of culture tubes where air might have been admitted. In all of the plants studied, and which were observed in this connection, it was concluded that branching of the root would occur whenever there was sufficient oxygen in the soil to permit root-growth. In certain species, however, there appeared to be a differential reaction in the oxygen relation as between the main root and the lateral roots. Such condition was observed in *Opuntia*, *Pisum*, and in *Zea* and may not be an uncommon occurrence. The reaction, however, does not appear to be in the same direction in all. Thus, the larger roots in *Opuntia* appear to continue growth for a longer time in a soil deficient in oxygen than the shorter roots. In certain species, however, as in *Zea*, the main root is less tolerant of a poor supply of oxygen than the smaller roots. There thus appears to develop a certain degree of adaptation to an oxygen deficiency. Whether this may not be related to possible downward movement of oxygen from the shoot to the root in such instances, rather than to a change in the tonus of the developing roots, was not determined.

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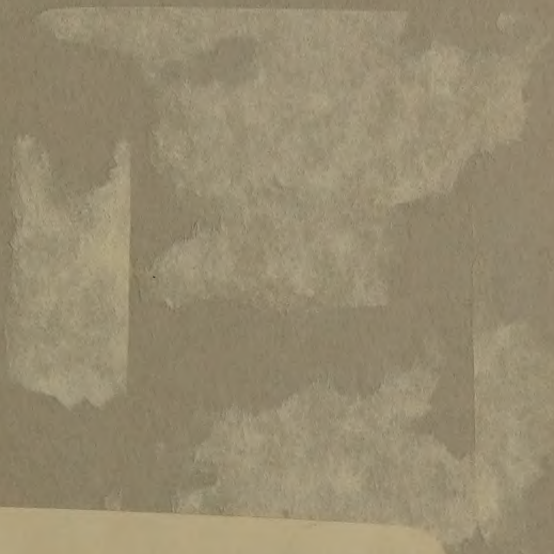

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